Medical Library JUL 19 1939

VOL. 24 JULY, 1939 No. 10

The Journal of Laboratory and Clinical Medicine

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Vol. 24, No. 10, July, 1939. Journal of Laboratory and Clinical Medicine is published monthly by The C. V. Mosby Company, 3523 Pine Blvd., St. Louis, Mo. Subscription Price: United States, Its Possessions, Pan-American Countries, Canada, $8.50 a year; Foreign, $9.50. Entered as Second Class Matter at Post Office at St. Louis, Mo., under Act of March 3, 1879.

The Journal of Laboratory and Clinical Medicine

VOL. 24 JULY, 1939 NO. 10

CLINICAL AND EXPERIMENTAL

HEMATOLOGIC STUDIES IN ACUTE INFECTIONS*

E. L. AMIDON, M.D., BURLINGTON, VT.

THE value of the white and differential counts in acute infections is accepted generally, as is also the "shift" as determined by Schilling. Some observers[1-4] have noted valuable informative evidence, as shown by the qualitative changes in the neutrophiles. The purpose of this work is to determine the relative value of these different methods.

Several methods of examination were made on the same blood, and the information thus gained was evaluated and compared. These methods were: (1) total white count; (2) differential count; (3) estimation of the percentage of young neutrophiles; (4) estimation of the percentage of neutrophiles containing "toxic granules"; (5) estimation of the percentage of neutrophiles showing evidence of severe damage; (6) calculation of the "index of resistance."

The percentage of young neutrophiles, the so-called "shift," was determined by the method of Farley.[5] The count is reported as the percentage of non-filamented neutrophiles per hundred leucocytes.

Probably Cesaris-Demil[6] was the first to describe the large, blue staining, peroxidase negative granules[7] in the cytoplasm of the neutrophiles. These are the "toxic granules" of Hirschfeld, Tuerk, Nageli, and many others. The result is reported as the percentage of neutrophiles containing such granules.

Ewing[8] in 1901 called attention to the evident degenerative changes sometimes seen in severe toxemias. Bodkin[9] in 1892 expressed the importance of such changes. Strumia[10] has chosen to separate the degenerated neutrophiles into three groups: (1) those cells showing only "toxic granules"; (2) those cells showing clumping of the cytoplasmic granules with vacuolization; (3) those

*From the Departments of Medicine and Pathology, University of Vermont College of Medicine.

Summary of thesis submitted as partial requirement for degree of M.S. in Medicine from the Graduate School of Medicine of the University of Pennsylvania.

Received for publication, December 19, 1938.

cells showing "coagulation necrosis" and edema, terminating in nuclear rupture. The cells classed as "degenerated" neutrophiles were those listed in Strumia's third group. The result is reported as the percentage of neutrophiles showing these degenerated changes.

The "index of resistance," as suggested by Wilson,[11] was calculated from the formula (T-10)-(P-70), in which T is the total white count in thousands and P is the percentage of neutrophiles. It apparently is of little significance when the white count is less than 10,000.

The above studies were done on fresh blood smears made on slides and immediately stained following the technique recommended by Strumia.[10]

The first studies were made on cases of acute appendicitis, counts being done on admission, and on the third and sixth hospital days. The interpretation of each count was compared then to the clinical course of the patient and the pathologic report of the appendix. Following these studies, other acute infections were studied in a similar manner. A total of 330 patients were studied and 550 examinations were made.

In order to compare these different examinations it was necessary to reduce the findings to common terms. Mendell[12] has established the numerical limits for the nonfilamented and "toxic-granular" count which seems acceptable for our use here. The interpretation of the lesion, as indicated by the percentage of degenerated cells, is our own choosing and may differ from that of other observers.

	NONFILAMENTED	"TOXIC GRANULES"	DEGENERATED
Mild infection	-16	-20	-10
Moderate infection	17-30	21-40	11-25
Severe infection	31-40	41-70	26-40
Grave infection	41 up	70 up	41 up

Fourteen of the patients studied had diagnoses of acute appendicitis, from whom gangrenous appendices were removed. These cases are listed in Table I with the blood counts, the interpretation, the pathologic diagnosis, and a few clinical notes.

TABLE I

GANGRENOUS APPENDICITIS

PATIENT	PREOPERATIVE				THIRD DAY				SIXTH DAY				COMMENT
	NF	"T.G."	Deg.	I-R	NF	"T.G."	Deg.	I-R	NF	"T.G."	Deg.	I-R	
1	G	G	G	- 9	G	G	S	- 5	G	G	S	- 2	Pelvic abscess
2	G	S	M	- 6	S	M	Mi	0	G	G	Mi	*	
3	M	S	M	- 5	S	G	M	- 2	S	S	M	*	
4	G	Mi	Mi	-15	G	G	N	*	G	G	N	2	General peritonitis
5	M	Mi	N	0	G	S	N	1	G	G	N	- 5	General peritonitis
6	G	Mi	M	-18	G	S	M	*	S	S	Mi	-16	General peritonitis
7	S	S	Mi	-10	M	S	N	5	M	G	Mi	*	
8	M	M	M	- 9	M	G	Mi	-12	M	M	M	*	Local peritonitis
9	G	G	Mi	3	G	G	N	- 3	G	G	Mi	- 7	Local Peritonitis
10	G	S	N	-16	G	S	Mi	-15	S	S	Mi	0	
11	G	M	G	(Lived only few hours after admission. General peritonitis)									
12	S	M	Mi	- 4	S	M	Mi	*	S	G	M	0	
13	G	G	S	*	G	G	S	*	G	G	G	*	General peritonitis
14	G	G	M	0	G	S	M	1	G	Mi	Mi	4	

NF = nonfilamented, "T.G." = "toxic granules," Deg. = degenerated, I-R = index of resistance.

G = grave, S = severe, M = moderate, Mi = mild, N = normal, * = white count below 10,000.

Comment.—The percentage of nonfilamented cells indicated the presence of an infection in all of the 14 cases, and in all roughly paralleled the severity of the process. The percentage of "toxic granules" failed to show such a process in 4 of the patients, and the percentage of degenerated cells was not abnormally elevated in 7. Where applicable, the "index of resistance" followed the clinical course closely.

From the foregoing it would appear that the nonfilamented cell percentage was the most helpful, while the percentage of degenerated cells was of the least value.

TABLE II

ACUTE APPENDICITIS NOT GANGRENOUS

PATIENT	PREOPERATIVE				THIRD DAY				SIXTH DAY				COMMENT
	NF	"T.G."	Deg.	I-R	NF	"T.G."	Deg.	I-R	NF	"T.G."	Deg.	I-R	
1	M	N	Mi	*	M	S	Mi	*	M	S	Mi	*	
2	G	S	M	-4	M	M	Mi	*	M	G	Mi	*	
3	S	S	Mi	-3	M	M	Mi	-12	M	S	N	*	Local peritonitis
4	Mi	M	N	*	M	S	N	*	M	M	Mi	*	
5	Mi	M	Mi	*	S	M	N	*	S	Mi	N	*	Phlebitis third day
6	N	Mi	N	*	M	M	Mi	*	M	S	N	*	
7	G	N	N	*	S	S	N	*	G	S	Mi	*	
8	M	M	Mi	*	Mi	S	N	*	M	Mi	N	*	

Comment.—In all of the 8 patients with acute appendicitis enumerated in Table II, the presence of an inflammatory process was indicated by the percentage of nonfilamented cells. The percentage of "toxic granules" indicated such a process in 6 of the patients. The percentage of degenerated cells was not sufficiently elevated to indicate any inflammation in 5 of the 8 patients.

In the group of 22 patients with appendicitis, 14 of which were gangrenous and 8 of which were mildly acute, the percentage of nonfilamented cells was elevated in 100 per cent, the percentage of "toxic granules" was elevated in 73 per cent, while the percentage of recognizable degenerated neutrophiles was elevated in 43 per cent.

TABLE III

PNEUMONIA

PATIENT	NONFILAMENTED				"TOXIC GRANULES"				DEGENERATED				INDEX RESISTANCE*	COMMENT
	Mi	Mo	S	G	Mi	Mo	S	G	Mi	Mo	S	G		
1				1				1		1				Died following day
2				3				3		1	2		- 6, -10	Died in 3 days
3		1	2					3		3			0, - 3	Discharged 15th day
4		3	2	2			3	4		5	2		- 5, 10	Discharged 20th day
5				3				3		1		2	-16, -24	Died in 4 days
6		2	2	3				7	1	5	1		- 7, 13	Discharged 13th day
7				6			2	4			2	4	-27,	Died on 8th day
8				5				5			4	1	- 4, -20	Discharged 41st day
9				1				1				1	- 3	Died day admitted
10			1	6			3	4		3	2	2	- 4, -15	Died on 7th day

*Extreme limits.

Comment.—In the 10 patients with pneumonia, 6 cases of which were fatal, the percentage of "toxic granules" indicated a grave process in all. The percentage of nonfilamented cells indicated a grave process in all of the 6 fatal cases, and one of moderate degree in all but one of the nonfatal cases. In much the same manner the percentage of degenerated cells indicated a grave process in 3 of the 5 fatal cases, and indicated a grave process in one of the 4 nonfatal cases.

It would thus seem that the percentage of nonfilamented cells was the most helpful and accurate index in this series, followed very closely by the percentage of degenerated cells. The percentage of "toxic granules" was of little help. Mention should be made of the fact that patient No. 8, who received a grave prognosis from both the nonfilamented and degenerated cells, received adequate dosage of sulfanilamide, and seemed clinically to be improved by it.

TABLE IV

MISCELLANEOUS INFECTIONS

PATIENT	NONFILAMENTED			"TOXIC GRANULES"			DEGENERATED			INDEX RESISTANCE*	DIAGNOSIS	COMMENT
	Mo	S	G	Mo	S	G	Mo	S	G			
1			2			2		2		-10, -18	Typhoid	Died on 4th day
2		1	2			3	1		2		Typhoid	Discharged 31st day
3			2	1		1	1	1		-12, -19	Streptococcic bacteremia	Died on 4th day
4	4	3	3	6	2	1	9	1		- 1, 12	Meningitis (pneumococcic)	Discharged 6th week
5			1			1			1	- 4	Meningitis (pneumococcic)	Died day of admission
6			3			3		1	2	- 1, -18	Erysipelas	Died on 4th day
7			2			2		2		- 6, -14	Bacterial endocarditis	Died on 6th day
8			2			2			2	- 4, - 3	Gangrenous stomatitis	Died day of admission
9			2			2	2			-12, -13	Ruptured ulcer (peritonitis)	Died 4 days later
10	2	1			1	2				- 7, 0	Acute cholecystitis	Discharged postop. 31st day
11		1	1		2		1	1		- 8	Pulmonary tuberculosis	Discharged 2 wk. later
12		1	1		2			2		- 5, 0	Meningitis (TB)	Died day following admission
13	1					1				2	Meningitis (TB)	Died day following admission
14		2				2	2			- 5, 0	Pyelonephritis	Discharged recovered
15		1		1			1				Hydronephrosis	Died day of admission
16	Mild		3	1	1		Mild		3	-18	Pregnancy (appendicitis?)	Discharged 9th day
17	2			1	1		1	1		-11, - 5	Appendicitis?	Discharged 10th day
18	1	2	20		2	16	2	3	13	-25, 20	Hemolytic streptococcus wound infection	Discharged 10th wk.
19		1	3		2	2	2	1		- 7, - 4	Appendicitis? Tonsillitis	Discharged 5th day
20	1	1	1			3	Normal		3	- 9, - 7	Salpingitis	Salpingectomy—uneventful recovery
21		1	3		1	3	3	1		- 9, 6	Staphylococcic bacteremia	Discharged 3½ months later

*Extreme limits.

Comment.—In the 21 cases of miscellaneous infections in Table IV, the information derived from each count is compared with the clinical findings in relation to the type and the severity of the process, together with the outcome. The count most closely corresponding to the clinical evidence, in our judgement, is listed as number 1, the other counts being graded 2 and 3, in the order in which they approach the first. The results are tabulated as follows:

Patient No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Nonfilamented	1	1	1	3	1	1	1	1	1	1	2	1	2	2	1	1	1	1	2	2	1
"Toxic granules"	1	3	2	2	1	1	1	1	1	2	1	2	1	3	2	2	2	1	2	1	1
Degenerated	3	2	3	1	1	1	2	1	3	3	3	2	2	1	2	1	1	1	1	3	1

It may be seen from the above that the percentage of nonfilamented cells indicated a process closely corresponding to our impression of the case clinically in 15 out of 21 cases (71 per cent). In the case of one patient, the conclusions derived from the percentage of nonfilamented cells were considered less accurate than those of the other counts. The percentage of "toxic granules" seemed to reflect the clinical condition accurately in 11 of the 21 cases (52 per cent). The conclusions derived from the percentage of degenerated cells seemed to be accurate in 11 of the 21 cases (52 per cent). In 6 of the listed cases the percentage of degenerated cells indicated a process which was considered the least accurate of the counts. In 6 cases the white count was not sufficiently elevated to calculate the "index of resistance," in 9 it seemed to follow the clinical course closely, and in 6 it was of questionable significance.

DISCUSSION

In estimating the value of these several methods of examination, the information gained must be balanced against the time, effort, and equipment required. The determination of the percentage of nonfilamented cells is relatively easy, requiring a small amount of time, no special technical skill, and appears to be subject to little individual variation. The counting of "toxic granules" is also relatively easy, requiring somewhat better staining, but again subject to little variation in individual examiners. The determination of the percentage of degenerated cells, on the other hand, is difficult. It requires good smears, a good stain, and very close examination of the neutrophiles, necessitating the expenditure of more time. With the best obtainable technique the individual variations must be large. It would appear that only in exceptional circumstances could hospitals incorporate this procedure into the routine laboratory examinations. Probably more skill and experience would produce better results, but from our experiences there would seem to be little reason for encouraging the adoption of such a procedure.

SUMMARY

In 22 cases of acute appendicitis, the percentage of nonfilamented cells was found to give more accurate information regarding the presence and severity of the process than the percentage of either "toxic granules" or degenerated cells.

The prognosis, as indicated by the percentage of nonfilamented cells in 10 cases of pneumonia, was more accurate than either of the other counts.

In 21 cases of miscellaneous infections, the percentage of nonfilamented cells indicated a condition more frequently in accord with the clinical findings than either of the other two.

CONCLUSION

For accuracy of information, speed, and ease of execution, it would appear that the percentage of nonfilamented cells was the most valuable qualitative examination in this group of infections.

The impression is gained from this study that the determination of degenerated cells requires a degree of training and technical skill not to be expected of the average hospital technician.

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The author's appreciation is due to Dr. R. C. Fuller and Dr. R. D. Towne for technical assistance, and to Dr. E. H. Buttles for his invaluable advice and counsel.

THE ANTACID PROPERTIES OF MAGNESIUM TRISILICATE IN NORMAL SUBJECTS AND PATIENTS WITH PEPTIC ULCERS*

PHILIP KURTZ, M.D., INDIANAPOLIS, IND.

MAGNESIUM trisilicate has recently received attention as a means of neutralizing excess gastric acidity (Mutch[1-3]). In the presence of hydrochloric acid a colloidal gel is formed with a neutralizing power, which is partly chemical and partly adsorptive. The initial phase of neutralization is rapid and the subsequent phase prolonged. The toxicity is low and danger of alkalosis is minimized by the fact that magnesium trisilicate is insoluble in distilled water and weak alkalies. An excess is, therefore, not absorbed, and regardless of the amount given, the gastric reaction will not go far below neutrality. This is a property which may be of clinical importance.

Mutch states that it does not disturb bowel motility and that it has a selective adsorbing power for certain bacterial toxins, putrefactive amines, and food poisons. Mann[4] reported a nine-hour fractional test meal on a patient with a gastric ulcer receiving 1 dram of magnesium trisilicate every hour. Free hydrochloric acid was absent in 17 of 19 samples. Mutch reported 7 normal subjects with fractional gastric analyses following a standard meal with and without 35 grains of magnesium trisilicate. He demonstrated that a substantial reduction in hydrochloric acid occurred.

Since there is often variation in the results of consecutive gastric analyses on normal persons following Ewald or alcohol meals, it was decided to test the antacid properties of magnesium trisilicate after administration of histamine acid phosphate, given subcutaneously. There is a question whether histamine stimulates the production of gastric enzymes,[5] but it is known to be a powerful acid secretagogue. Therefore, it seems adequate as a test meal for demonstrating antacid properties of magnesium trisilicate.

METHODS

Fourteen of the subjects were normal young adult white males, 21 to 33 years of age. Three of the subjects were white males, 23 to 45 years of age, with peptic ulcers previously demonstrated by x-ray. All 3 had typical symptoms and had not been treated for several days.

The subjects were in the postabsorptive state. A Rehfuss tube was passed and the fasting gastric contents were completely aspirated. Then they received 0.5 mg. histamine subcutaneously. This was not more than five minutes before the administration of water or water and magnesium trisilicate. On the control day, 50 c.c. tap water were administered by the Rehfuss tube, and on the

*From the Indianapolis City Hospital, Indianapolis.
Received for publication, August 27, 1938.
Magnesium trisilicate as Trisomin, Lilly was kindly furnished by Eli Lilly and Co., Indianapolis.

test day, 50 c.c. tap water plus 15 grains magnesium trisilicate were given. Ten cubic centimeters of gastric juice were withdrawn three times at thirty-minute intervals following the histamine injection.

The control day preceded the test day in 9 normal cases and all 3 of the ulcers. The control day followed the test day in 5 of the normal cases.

TABLE I

THE AVERAGE TITRATABLE ACID AND pH OF GASTRIC JUICE OF 14 NORMAL SUBJECTS AND 3 PATIENTS WITH PEPTIC ULCER, USING HISTAMINE STIMULATION WITH AND WITHOUT ADMINISTRATION OF 15 GRAINS OF MAGNESIUM TRISILICATE

	TÖPFER'S REAGENT		PHENOL RED		pH	
	WATER	WATER + MAG. TRIS.	WATER	WATER + MAG. TRIS.	WATER	WATER + MAG. TRIS.
Normal Subjects						
	acid per cent		*acid per cent*			
Fasting	10.2	13.1	15.1	20.2	4.46	3.84
30′	37.6	9.4	42.3	14.3	1.94	3.24
60′	63.5	26.9	69.2	33.5	1.26	1.93
90′	52.3	22.3	61.3	31.6	1.36	2.29
Patients With Peptic Ulcer						
	acid per cent		*acid per cent*			
Fasting	36.3	19.2	47.0	29.5	1.63	2.03
30′	52.6	22.6	57.2	27.2	1.23	2.43
60′	92.3	50.3	96.5 Av. of 2	54.3	1.13	1.40
90′	74.3	30.0	98.5 Av. of 2	39.7	1.13	1.66

Determination of pH by the colorimetric method of Brown[6] and titration of 1 c.c. of filtered gastric juice to pH 7.0 with 0.01 N sodium hydroxide, using phenol red as the indicator as recommended by Helmer and Fouts,[7] were done on each sample without delay. Titration of 1 c.c. filtered gastric juice with 0.01 N sodium hydroxide, using Töpfer's reagent as the indicator, was also done for comparison. The titration figures were multiplied by 10 to give the number of cubic centimeters of 0.1 N hydrochloric acid per 100 c.c. gastric juice or units of acid per cent.

RESULTS

Table I shows that 15 grains of magnesium trisilicate reduced the average titratable acidity of the gastric juice of 14 normal subjects 28.0 acid per cent at the thirty-minute interval, 35.7 acid per cent at sixty minutes, and 29.7 acid per cent at ninety minutes. This checks well with the decrease noted when using Töpfer's reagent as the indicator: 28.2 acid per cent at thirty minutes, 36.6 acid per cent at sixty minutes, and 30.0 acid per cent at ninety minutes. The pH was correspondingly increased.

A similar reduction in titratable acidity was found in 3 cases of peptic ulcer (Table I).

The ninety-minute samples of each person tested showed a reduction in acidity (the average decrease of all 17 at ninety minutes was 32.5 acid per cent) despite the fact that an appreciable amount of magnesium trisilicate was neces-

sarily removed with the thirty- and sixty-minute samples of gastric juice withdrawn for analysis. It made no difference whether the control day preceded or followed the test day.

CONCLUSIONS

The efficiency of magnesium trisilicate as a gastric antacid has been demonstrated following histamine stimulation in 14 normal subjects and 3 patients with peptic ulcer.

Reduction in free acid was still marked ninety minutes after administration of 15 grains of magnesium trisilicate.

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EFFECT ON BACTERIOPHAGE OF PRONTYLIN, PRONTOSIL, SULFAPYRIDINE, AND OTHER ANTISEPTICS AND DYES USED IN SURGICAL PRACTICE*

Helen Zaytzeff-Jern, M.D., and Frank L. Meleney, M.D.
New York, N. Y.

INTRODUCTION

SURGICAL infections are frequently caused by two or more pathogenic organisms each requiring a different method of attack. For the staphylococcus, the most common causative organism of wound infection, potent bacteriophages have been successfully employed in recent years.[37-39] The colon bacillus and *B. pyocyaneus*, which are commonly found in postoperative cystitis and in wounds infected by intestinal organisms, often promptly disappear under potent bacteriophage treatment. Nevertheless, many wounds are infected with organisms for which no potent bacteriophages have yet been found. Among these are the large groups of streptococci. The hemolytic streptococcus which is often found in combination with *Staphylococcus aureus* has heretofore been an infective agent causing great concern to the surgeons. But recently sulfanilamide[40-42] and zinc peroxide[43-45] have been shown to be effective agents against it; the

*From the Bacteriological Research Laboratory, the Department of Surgery, Columbia University, New York.
Received for publication, September 6, 1938.

former as an internal medicament and the latter as a local applicant. However, it has been found that these chemicals have little or no effect on staphylococci and certain other pathogenic bacteria. The question arises whether they, as well as other specific or general antiseptics, can be used in combination with bacteriophage to combat mixed infections. The present paper attempts to answer this question.

LITERATURE

There is a long list of reports on the effect of antiseptics on bacteriophage. The literature on this subject is too voluminous to review here but reference is given in the bibliography.[1-36] Suffice it to say that these reports have shown that antiseptics in general inhibit or destroy bacteriophage. Some authors have shown that there is a differential action of certain antiseptics on different types and on different races of phage within the same type.

EXPERIMENTAL

Our study was undertaken for the purpose of determining whether certain antiseptics could be used with our phages in the treatment of mixed infections.

The antiseptics and dyes frequently employed on the surgical service of the Presbyterian Hospital, New York, are (1) prontylin (sulfanilamide); (2) 2.5 per cent prontosil; (3) Dakin's solution; (4) 2 per cent and 4 per cent boric acid; (5) red wash (1 per cent zinc sulfate); (6) 5 per cent aluminum acetate; (7) 95 per cent, 10 per cent, 5 per cent, and 0.5 per cent carbolic acid; (8) 1:10,000 silver nitrate; (9) 10 per cent argyrol; (10) 1 per cent acetic acid; (11) 25 and 70 per cent alcohol; (12) 2 per cent mercurochrome; (13) 1:1,000 and 1:10,000 merthiolate; (14) 1:500 and 1:5,000 metaphen; (15) 3.5 per cent iodine; (16) 1:500 azochloramide; (17) 1:1,000 hexylresorcinol; (18) 1 per cent to 10 per cent zinc peroxide; (19) 1:2,000 methylene blue; (20) 2 per cent gentian violet. The surgical solutions include (21) physiological saline (0.85 per cent), 1 per cent and 5 per cent saline; (22) Ringer's solution; (23) 5 per cent dextrose solution in distilled water and 5 per cent dextrose in Ringer's solution; (24) 1 per cent novocain. The recent discovery of sulfapyridine (derivative of sulfanilamide) and its wide use in infections caused by a variety of pathogenic microorganisms made it necessary to carry through an additional series of experiments on the effect of this substance upon the action of bacteriophage.

All of our experiments were carried out with our staphylococcus, colon bacillus, and *B. pyocyaneus* bacteriophages and their corresponding bacteria. We employed two staphylococcus phages: (A) polyvalent phage "BK" of Gratia and (B) stock staphylococcus phage mixture, both forming plaques on solid media in the seventh decimal dilution (10^{-7}). *Staphylococcus aureus* "strain F" was used for their propagation in the course of the experiments. Polyvalent *B. coli* phage "MK" of MacNeal formed plaques on solid media in the sixth decimal dilution (10^{-6}). Colon bacillus "strain I" was used for its propagation. (A) polyvalent pyocyaneus stock mixture phage of MacNeal which formed plaques on solid media in the sixth decimal dilution (10^{-6}) was used with *B. pyocyaneus* "strain D."

TECHNIQUE

In order to test as nearly as possible the actual concentration of the antiseptic commonly used for treatments, we employed 1 c.c. of ten times concentrated savita broth, to which was added 9 c.c. of antiseptic of the desired strength. Prontylin and sulfapyridine were dissolved directly in 1 per cent savita broth and zinc peroxide (Du Pont medicinal grade Z.P.O.) was suspended in savita broth as well as in distilled water. The effect of the surgical solutions was studied both with and without savita broth. One-tenth cubic centimeter of staphylococcus phage or 0.25 c.c. of *B. coli* or *B. pyocyaneous* phages was added to 10 c.c. of media and enough bacteria to make a final suspension of 50 million per c.c. throughout the experiments (see Figs. 1 and 2).

Our experiments differed from those of previous workers in that we demonstrated the complete inhibition or the diminution of the titer of the phages both in liquid and on solid media. The effect of sulfanilamide, sulfapyridine, and zinc peroxide is shown for the first time.

RESULTS

The results of our experiments are presented in four groups. The first group embraces 11 antiseptics which destroyed staphylococcus, *B. coli*, and *B. pyocyaneus* bacteriophages and bacteria equally well within twenty-four hours. This group includes 95 per cent and 10 per cent carbolic acid, 10 per cent argyrol (solargentum), 70 per cent alcohol, 2 per cent mercurochrome, 3.5 per cent iodine, 1:500 and 1:5,000 dilutions of metaphen in water, 1 per cent acetic acid, 5 per cent carbolic acid and 5 per cent aluminum acetate.

TABLE I

TITER AND LYTIC ABILITY OF STAPHYLOCOCCUS, B. COLI, AND B. PYOCYANEUS PHAGES AFTER 24-HOUR CONTACT WITH THE ANTISEPTICS WHICH DIFFERENTIALLY OR PARTIALLY INACTIVATED THE PHAGES

	STAPH. PHAGE		B. COLI PHAGE		B. PYOCYANEUS PHAGE	
	TITER	LYTIC ABILITY	TITER	LYTIC ABILITY	TITER	LYTIC ABILITY
Dakin's solution	0	0	0	0	4	4+
1:1,000 Merthiolate	0	0	0	0	4	4+
2% Gentian violet	0	0	u	0	u	0
1:500 Azochloramide	0	0	2	4+	1	4+
10% Zinc peroxide	0	0	3	-	1	-
1:2,000 Methylene blue	0	0	2	0	4	4+
25% Alcohol	0	0	3	3+	3	4+
Red wash 1 per cent zinc sulfate	0 or 1	0	3	0	4	±
1:10,000 Silver nitrate	0 or 1	0	3	4+	4	4+
1:1000 Hexylresorcinol	0	0	4	4+	4	4+
1% Zinc peroxide	0	0	4	-	4	-
1% Novocain	0 or 2	0	4	4+	4	4+
1:10,000 Merthiolate	0	0	5	4+	-	-
0.5% Carbolic acid	3	-	5	-	-	-
4% Boric acid	2 or 3	4+	3 or 4	4+	3 or 4	4+
2% Boric acid	3 or 4	4+	4	4+	4	4+
Savita control	7	4+	6	4+	6	4+

0 = Inability to recover bacteriophage (titer).
u = Recovery of phage from undiluted filtrate.
1 up to 7 = 10^1 up to 10^7 phage titer on solid medium.
4+ = Complete visible lysis in broth.
3+ = Partial lysis.
0 = No lysis.
- = Not tested.

The second group included those found to have a differential or partially lethal effect on our phages, as shown on Table I. This includes, in the order of their deleterious effect: Dakin's solution, 1:1,000 merthiolate, 2 per cent gentian violet, 1:500 azochloramide, 10 per cent zinc peroxide, 1:2,000 methylene blue, 25 per cent alcohol, red wash (1 per cent zinc sulfate), 1:10,000 silver nitrate, 1:1,000 hexylresorcinol, 1 per cent zinc peroxide, 1 per cent novocain, 1:10,000 merthiolate, and 4 per cent boric acid.

Most of the antiseptics of this group produced complete destruction of staphylococcus bacteriophage and only partial destruction of *B. coli* and *B. pyocyaneus* phages. *B. pyocyaneus* phage in general proved to be more resistant to the effect of antiseptics than *B. coli* phage.

Of particular interest to us was the effect of 1 per cent novocain which was found to possess a highly destructive action against staphylococcus phage diminishing the titer from 10^7 to 10^2. There was also a decrease of titer from 10^6 to 10^4 of both *B. coli* and *B. pyocyaneus* phages.

Five-tenths per cent carbolic acid and 2 per cent and 4 per cent boric acid represent the weakest among the commonly used surgical antiseptics. They produced only a partial destruction of staphylococcus, *B. coli*, and *B. pyocyaneus* phages.

TABLE II

TITER AND LYTIC ABILITY OF STAPHYLOCOCCUS, B. COLI, AND B. PYOCYANEUS PHAGES AFTER 24-HOUR CONTACT WITH PRONTYLIN, PRONTOSIL, AND SULFAPYRIDINE

	STAPH PHAGE		B. COLI PHAGE		B. PYOCYANEUS PHAGE	
	TITER	LYTIC ABILITY	TITER	LYTIC ABILITY	TITER	LYTIC ABILITY
1:100 and 1:1,000 Prontylin	7 or 6	4+	6	4+	5 or 6	4+
1:10,000 and 1:1,000,000 Prontylin	7	4+	6	4+	6	4+
2.5% Prontosil	4	0	6	4+	6	4+
1%, 0.5% and 0.1% 1:10,000 and 1:33,000 Sulfapyridine Prontosil	7 or 6	4+	6	4+	6	4+
Savita control	7	4+	6	4+	6	4+

4, 6, 7 = $10,^4$ $10,^6$ and 10^7 phage titer on solid medium.
4+ = Complete visible lysis in broth.
0 = No lysis.

Table II presents the third group which shows results of the effect on phage of various dilutions of prontylin (sulfanilamide) and prontosil. In order to approach as nearly as possible the actual concentration of sulfanilamide in the body, a wide range of dilutions from 1:100 to 1:1,000,000 were used for the experiment. None of these dilutions was found to exert any deleterious effect on the titer or the lytic ability of *B. coli* phages, as compared with savita controls. This was found to be the case after twenty-four hours of incubation, and again after one week and one month of refrigeration. A 1 per cent dilution of sulfanilamide was found to produce an occasional diminution of the titer of staphylococcus phage from 10^7 to 10^6 and of *B. pyocyaneus* phage from 10^6 to 10^5. There was complete visible lysis by the treated phages in liquid medium. A 1 per cent dilution of sulfanilamide was also found to possess only

a slight bacteriostatic effect on all three types of bacteria, and this was noticeable only during the first few hours of incubation. One-tenth per cent and higher dilutions did not exert any visible effect either on phages or bacteria. Results with sulfanilamide in savita broth which was heated up to 50° C. were very similar.

The effect of sulfapyridine was studied in dilutions 1:1,000, 1:10,000, and 1:33,000. These dilutions represent the range of concentration which sulfapyridine usually reaches in the blood of patients treated with this agent. None of these dilutions of sulfapyridine was found to exert any deleterious effect upon the lytic ability of staphylococcus, *B. coli*, and *B. pyocyaneus* bacteriophages. On the contrary, there was an indication that sulfapyridine enhanced the lytic effect of staphylococcus and *B. coli* phages. This study is to be presented in a separate publication. However, the titer of the bacteriophages propagated in the presence of sulfapyridine usually remained unchanged or fell slightly.

There was a definite bacteriostatic effect against *B. coli* and *B. pyocyaneus*, which was most pronounced within the first six hours. However, after twenty-four hours, the difference in degree of bacterial growth in tubes containing sulfapyridine and the controls was only slight or completely absent. No bacteriostatic effect was observed toward cultures of *Staphylococcus aureus*.

Compared with other dyes previously noted the antiseptic power of 2.5 per cent prontosil showed the least deleterious effect. Twenty-four-hour contact of 0.1 c.c. of staphylococcus phage with 10 c.c. of 2.5 per cent prontosil resulted in lowering the titer of the phage from 10^7 to 10^4 and the lytic ability of the treated phage was completely inhibited. However, when 1 c.c. of phage was taken instead of 0.1 c.c., the titer of the phage was reduced only from 10^7 to 10^6; in other words, was practically unaltered. Twenty-four-hour contact of 2.5 per cent prontosil with *B. coli* and *B. pyocyaneus* phages brought no change of titer or lytic ability to these phages. There was no visible effect on the development of staphylococcus, *B. coli*, or *B. pyocyaneus* organisms. One per cent, 0.5 per cent, and 0.1 per cent dilutions of prontosil were likewise found to produce no visible changes in staphylococcus, *B. coli*, and *B. pyocyaneus* phages, or their homologous bacteria. In fact, titration of staphylococcus phage after twenty-four-hour contact with 1 per cent and 0.5 per cent prontosil showed the titer to be slightly higher than that of the savita control, and the lysis in liquid medium took place a little before the savita control. The possibility of an adjuvant action of these dilutions on bacteriophage is being studied and will be reported later.

The surgical solutions are included in the fourth group. Bacteriophages prepared in savita broth as well as in distilled water containing 1 per cent saline (sodium chloride) instead of 0.5 per cent, with one exception, differed in no respect from the control phages. The same was true for Ringer's solution, 5 per cent dextrose in distilled water, and 5 per cent dextrose in Ringer's solution.

There was only partial lysis of the staphylococcus in 5 per cent saline (sodium chloride) in savita broth, and the titer of the phage was found to be 10^6. On the other hand, there was complete lysis with *B. coli* and *B. pyocyaneus* phages, although the titer of the phages was diminished in both cases to 10^4.

DISCUSSION

In this study we have attempted to find out what effect certain antiseptics and other dressing solutions, commonly used in surgical practice, had upon our potent staphylococcus, *B. coli,* and *B. pyocyaneus* bacteriophages. We have included a number of surgical solutions employed locally or parenterally, and novocain, which is sometimes used as a preliminary medication.

Comparing the results of previous workers with our own, we find a number of apparent discrepancies. Some of these seem to be due to differences in technique, such as variations in the medium in which phage is brought into contact with the antiseptic, its chemical reaction and its amount, as well as the time and temperature at which the experiments are carried out. But the chief discrepancy is probably due to the different types and the potency of the phages employed.

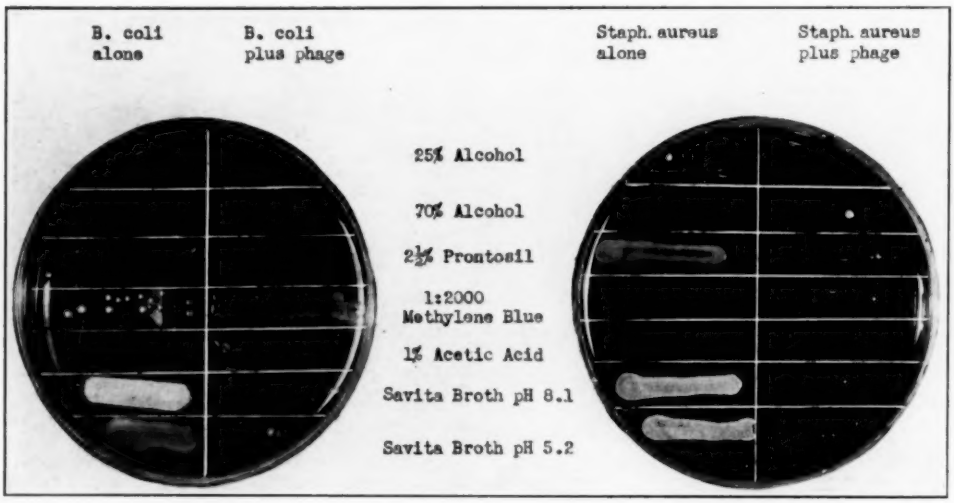

Fig. 1.—The comparative effect of various antiseptics on staphylococcus and *B. coli* phages and their corresponding bacteria after contact with 25 per cent and 70 per cent alcohol, 2.5 per cent prontosil, 1:2,000 methylene blue, 1 per cent acetic acid, and savita broth of pH 8.1 and 5.2. It is seen that 25 per cent and 70 per cent alcohol, and 1 per cent acetic acid destroyed staphylococcus and *B. coli* organisms. Methylene blue almost completely inhibited the growth of staphylococcus, but a few colonies of *B. coli* survived. Two and one-half per cent prontosil did not prevent the normal growth of either bacteria, but interfered with the lytic action of staphylococcus bacteriophage, as evidenced by the partial growth on the plate. There was no apparent effect on *B. coli* phage. A few colonies of growth from savita phage control of pH 5.2 indicate that it is affected by the low pH of the medium as contrasted with no growth from the pH 8.1 control tube.

Our work has been in general agreement with the previous reports to the effect that bacteriophage is susceptible to, and is destroyed by, certain of the stronger antiseptics, and that there is a differential susceptibility to some of the weaker antiseptics, the staphylococcus phages being regularly more susceptible than the *B. coli* phages. We have also shown that the latter in turn are more susceptible than the *B. pyocyaneus* phages.

None of our experiments has thrown any light upon the reason for this differentiation. It is well known, of course, that different species of bacteria vary in their susceptibility to antiseptics and to a less degree different strains within the same species vary. The analogy may be an indication that the bac-

teriophages are biologically similar to bacteria and behave more like living organisms in this respect than chemical reagents or even enzymes. It is of some interest that in general the bacteria are more susceptible than their corresponding phages. Potency does not seem to run parallel with susceptibility, because the two staphylococcus phages differed in potency but were similar in susceptibility. Also, the staphylococcus phages were both more polyvalent and more potent than the *B. coli* phages although the latter were more resistant. These in turn were more potent than the more resistant *B. pyocyaneus* phage mixture.

We have observed that the staphylococcus phages do not propagate as rapidly at low pH levels as the other two phages, and it may be that the low pH of most of the antiseptics may play a part in the easier destruction of the staphylococcus phages. However, staphylococcus phages were also more susceptible to the zinc peroxide suspensions which are on high pH levels. This agrees with Sierakowski and Zabludowska[36] who believe that the high pH ranges are also inhibitors of staphylococcus phage. They showed that staphylococcus phage was killed at pH 9.45, while *B. coli* phage was destroyed at pH 10.3. The chief dis-

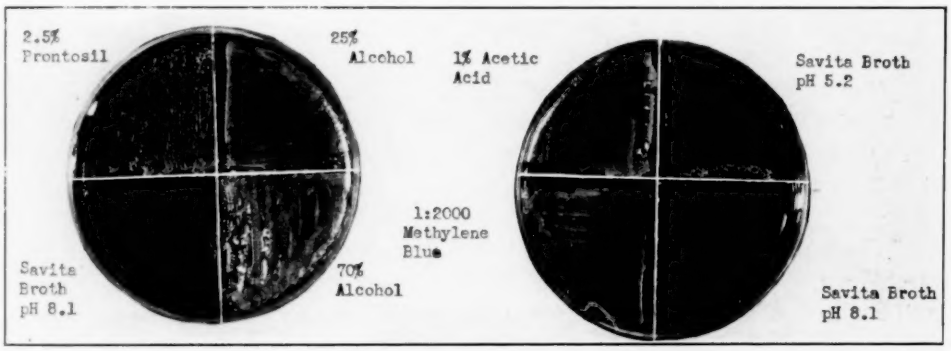

Fig. 2.—The potency of staphylococcus phage as estimated by titration on agar plates. Normal growth of staphylococcus on quadrants inoculated with bacteria and with a loopful of undiluted phage filtrate after contact with 25 per cent and 70 per cent alcohol, 1 per cent acetic acid, and 1:2,000 methylene blue show that bacteriophage is completely inactivated. Numerous plaques of phage replacing bacterial growth as the result of phage action after contact with 2.5 per cent prontosil show that bacteriophage is present but contains a smaller number of corpuscles than savita phage control. Phage grown in savita broth of pH 8.1 obviously contains a larger number of corpuscles causing greater destruction of bacteria than savita control of pH 5.2. The dark area on the methylene blue quadrant is the result of the coloration of the agar.

crepancies in our results as compared with those of other workers have been in connection with the dyes methylene blue, metaphen, and merthiolate, where there has been a difference of opinion expressed by a number of authors. These discrepancies may be explained by variations in the susceptibility of different races of bacteriophage or by differences in the technique employed in the tests. This is of some practical importance because commercial phages which we studied had been "preserved" with merthiolate, and we were unable to propagate them, indicating that they had not been preserved but killed by the antiseptic.

Our results seem to indicate that certain of the weaker antiseptics may have a dual action on bacteriophage: first, an inhibition of propagation and second, an interference with its lytic action. By our tests, which have been checked on both liquid and solid media, we have frequently shown that the titer has been considerably lowered by the antiseptic, in other words, that propagation has

been delayed or stopped altogether while the surviving amounts of phage were fully capable of producing complete lysis. The explanation for this is not clear.

We have felt that in these experiments the value of determining the presence of phage on solid media was more important than the lytic test in liquid media. Frequently we have demonstrated the presence of plaques on plates when the corresponding dilutions of phage failed to clear the media. This is probably due to the fact that the optimum relationship between the numbers of bacteria and phage corpuscles did not exist, because potent phage could always be recovered from the plaques developing on the plates. With certain phages the clearing mechanism in liquid media cannot function unless there is a fairly high number of bacteriophage corpuscles present.

The most important part of our study, and one which has not been reported by other authors, has been the observations of the effect of prontylin, sulfapyridine, and prontosil. Our finding that neither of these potent "streptococcicides" interferes with phage action makes it possible to combine the use of sulfanilamide and sulfapyridine with phage in those mixed infections which are caused by the hemolytic streptococcus combined with staphylococcus or *B. coli*, and we have used this combined method with gratifying results in a number of instances.

One of our experiments suggested that diluted prontosil might even have an adjuvant action on bacteriophage as the titer was higher after contact with 0.5 per cent and 1.0 per cent prontosil. Sulfapyridine, on the other hand, although it occasionally decreased the bacteriophage titer, definitely increased the lytic activity of bacteriophage. This action will be studied further.

The only other antiseptics which will permit this procedure are 2 per cent and 4 per cent boric acid, which are of questionable antiseptic value. Figs. 1 and 2 also indicate clearly that prontosil has little or no direct antiseptic action on the organisms which we have tested.

One per cent prontylin (which is considerably more concentrated than it ever is when it reaches the lesion in the human body) seemed to produce some inhibition of growth of staphylococcus for the first four hours in liquid media, but did not prevent ultimate growth.

The complete inhibition of lysis by staphylococcus phage after contact with 2.5 per cent prontosil (which had no such effect on the *B. coli* and *B. pyocyaneus* phages) is another example of differential action which we have not been able to explain.

CONCLUSIONS

1. Antiseptics in general cannot be used with bacteriophage in the treatment of mixed infections. Zinc peroxide is no exception to this rule.

2. Sulfanilamide, sulfapyridine, and prontosil may be used with bacteriophage in the treatment of mixed infections.

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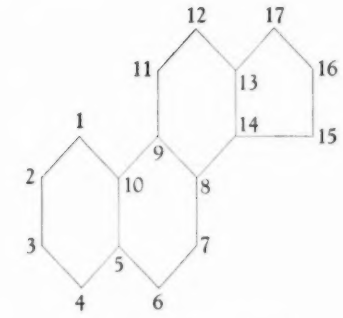
THE RATIONALE OF THE USE OF TESTOSTERONE PROPIONATE IN FUNCTIONAL UTERINE BLEEDING AND DYSMENORRHEA*

H. S. Rubinstein, M.D., Baltimore, Md.

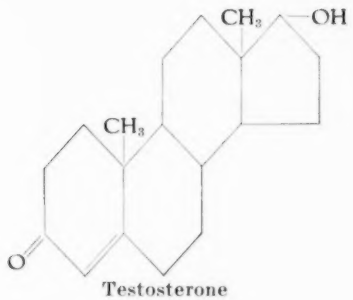
IT HAS recently been observed that testosterone propionate when administered in adequate dosage to patients with functional uterine bleeding or functional dysmenorrhea[1-4] leads to cessation of the bleeding and to an alleviation of the pain. It is difficult in the present state of knowledge to explain exactly why the male sex hormone favorably influences such bleeding and pain. Chemically testosterone propionate is quite similar in structure to progesterone[5] (Fig. 1). It is known that normally progesterone becomes an important factor after ovulation when further follicle effect is held in abeyance. One is, therefore, led to assume that suppression of overactivity of the Graafian follicle is an important factor in maintaining a normal menstrual flow. This concept suggests itself particularly when one realizes that persistent follicle cysts are frequent accompaniments of menorrhagic states and that functional dysmenorrhea has frequently been associated with excessive estrogen concentrations in blood and urine.[6, 7] Progesterone has been found to retard follicular maturation and to inhibit ovulation.[8]

Experiments conducted in this laboratory suggest that testosterone propionate has a similar effect. When testosterone propionate (Perandren, Ciba) was given to immature female rats 21 days of age in 1 mg. daily intraperitoneally adminis-

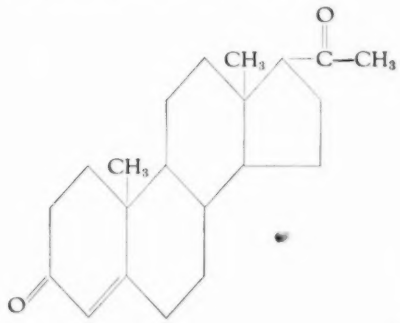
*From the Laboratory for Research, Surgical Division, Sinai Hospital.
Received for publication, November 8, 1938.



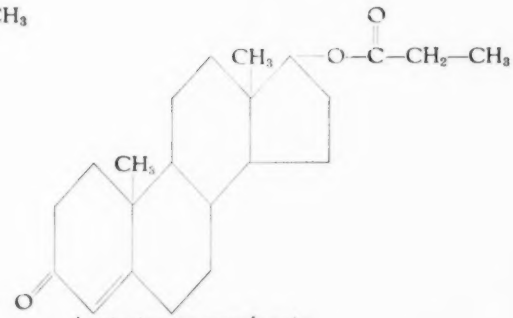
Phenanthrene nucleus
The numbers refer to carbon atoms.



Testosterone



Progesterone



Testosterone propionate
(4.4 × potency of testosterone)

Fig. 1.—Showing the close chemical relationship between testosterone propionate and progesterone. Both contain oxygen on carbon atom No. 3, a double bond between atoms 4 and 5, and CH_3 groups at atoms 10 and 13. Thus the basic ring structure is similar. The difference is found in the grouping on the No. 17 carbon.

tered doses, it was found that notwithstanding the fact that premature vaginal opening resulted at 30 days of age in test animals as compared to 38 days for the controls, the vaginal smears at the time of opening showed only a proestrus picture.[9] When sacrificed forty-eight hours later (32 days of age), their ovaries showed no evidence of ovulation. As a matter of fact, the ovaries of the test animals were smaller and the follicles were less mature than those found in the control (Fig. 2). In another group of animals in which similar injections were begun at 21 days of age and continued to the age of 67 days, it was found that the vaginas of this treated group also opened at 30 days of age, again displaying a proestrus smear, while vaginal opening in the controls occurred on the thirty-eighth day, at which time these animals also showed proestrus smears. Daily vaginal smears taken in all animals from the time of vaginal opening to the sixty-seventh day disclosed a normal five to six day complete estrus cycle in the controls while the test animals showed either diestrus or proestrus pictures. At autopsy the ovaries of the controls were again found to be larger than those of the test animals and were seen to contain large corpora lutea. No evidence of ovulation or luteinization was found in the ovaries of the injected animals (Fig. 1). This suggests a suppression of ovulation in the test animals as a result of repeated injections of testosterone propionate.

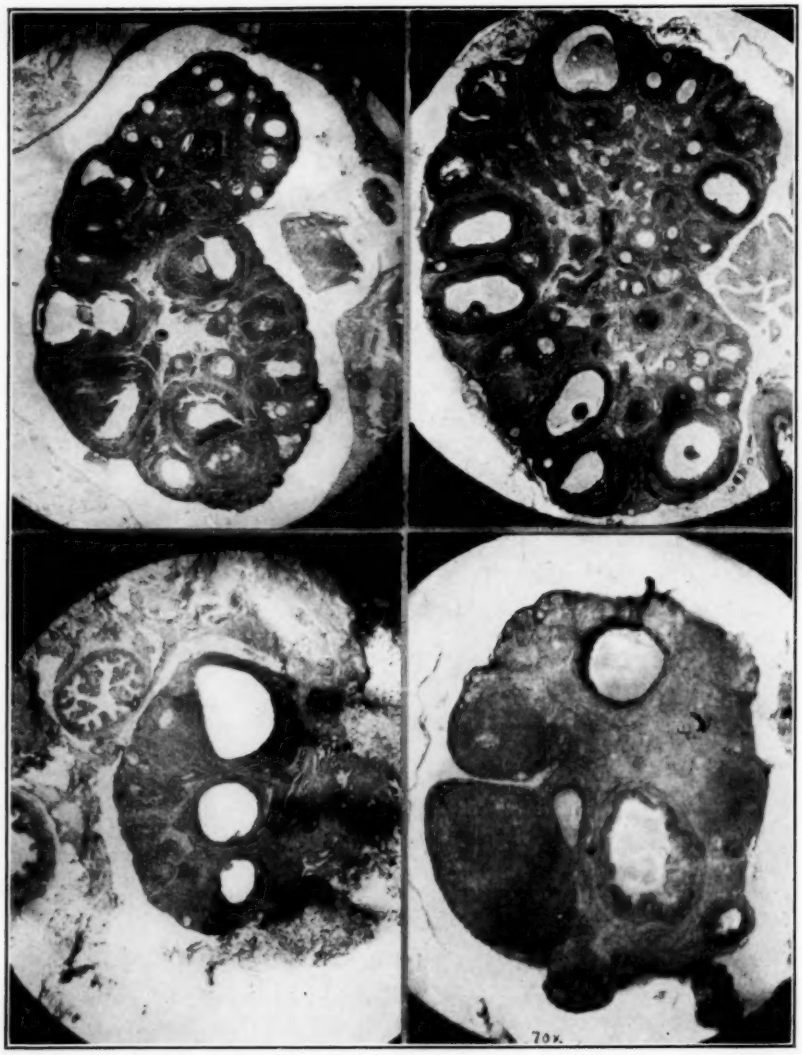


Fig. 2.—Comparison of ovaries of animals (rats) treated with testosterone propionate and their controls (magnification ×70). *A*, Ovary of 32-day-old treated rat, obtained forty-eight hours after artificially induced vaginal opening. The ovary is smaller and the follicles are at a lower stage of development than that seen in the control. No corpora lutea are visible. *B*, Ovary of 32-day-old control rat obtained while vagina was still unopened. The ovary is relatively large and the follicles appear highly developed. No corpora lutea are visible. *C*, Ovary of 67-day-old treated rat. Ovary is relatively small, no definite corpora lutea can be distinguished. *D*, Ovary of 67-day-old control. The specimen is twice the size of that seen in the treated animal. Several huge corpora lutea are present; follicle maturation is progressing normally.

Hence, it is possible that the similarity in chemical structure between testosterone propionate and progesterone may explain the progesterone-like effect of the substance used.

It will be noted from previous reports[3, 4] that the dosage which was successful in our cases was much smaller than that recently reported by others.[1, 2]

With doses of 500 mg. or more (using 15 mg. intramuscularly every other day), Loesser (1938) noted a suppression of menstruation which persisted for six to ten weeks after cessation of injections. Foss (1938) also observed amenor-

rhea following the use of 100 mg. daily. It appears that in spite of the fact that these authors noted no untoward effects following their large dosage, smaller amounts are more physiologic since menses are more normally established and maintained.

The cause of amenorrhea following injections of large doses of testosterone is probably inhibition of the pituitary gland with a suppression of output of pituitary gonadotropic substance.[10]

CONCLUSION

The rationale of the use of testosterone propionate in the treatment of functional uterine bleeding and functional dysmenorrhea is considered. It is felt that, due to its close chemical relationship to progesterone, testosterone propionate inhibits follicular maturation and ovulation and locally leads to relaxation of the myometrium. In addition in very large doses it inhibits the gonadotropic secretions of the anterior pituitary gland.

Estrogenic concentration is, therefore, diminished. This favors uterine relaxation and checks the continuation of the interval endometrium, thus altering the two common immediate causes of excessive pain and bleeding.

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The author gratefully acknowledges the cooperation of Ciba Pharmaceutical Products, Inc., for partially defraying the expenses involved in this study.

EXPERIENCE WITH ZINC INSULIN CRYSTALS*

CHARLES M. LEVIN, M.D., ELMER A. KLEEFIELD, M.D., AND
FRANK A. LUCIANO, M.D., JAMAICA, N. Y.

THE advent of protamine zinc insulin was a distinct advance in the treatment of diabetes mellitus. The diabetic who required insulin became more fortunate. His insulin administration was reduced to one injection per day, and the total amount of insulin required was often less than that of standard insulin. In many instances the food intake, particularly carbohydrate, could be increased above the previous allowance, and a certain amount of variation in the feeding time schedule was permissible. On the whole, the outlook for the diabetic was a happier one.

However, with the continued use of protamine zinc insulin in medium and severe diabetic patients, disturbing problems arose in the control of many of these individuals. Situations similar to those met by Ralli[1] and others were encountered. Commonly, of course, protamine zinc insulin when given alone did not always control early and forenoon hyperglycemia and glycosuria. On the other hand, the failure of a given supplement of standard insulin to control adequately and consistently the blood sugar fluctuations during this period was not rare. Some patients varied in their regulation from day to day to such an extent that some authorities wondered whether or not hyperglycemia and glycosuria were of any importance under these circumstances. We are in accord with Kepler[2] who states: "Nevertheless simply because we cannot always achieve a sugar free urine with the use of protamine zinc insulin, we as physicians are not justified in abandoning our former position that consistently sugar free urine is the 'sine qua non' in the treatment of diabetes." Wilder[3] also has said: "The degree of glycosuria under these circumstances may be harmless but until we have proof of its harmlessness we must insist on its control."

In the second place qualitative and quantitative rearrangements in the diet in order to meet the challenge of varying blood sugar levels became hardships to many individuals whose eating habits and working hours made such changes uncomfortable or inconvenient. For many juvenile diabetics the late evening feedings recommended were sometimes difficult problems. Discussing various types of dietary formulas for diabetics on protamine zinc insulin, Wilder[4] says: "These procedures undoubtedly are useful in tricky cases. I object to them for routine management because of the inconvenience they cause the patient. So far as possible we should strive to interfere as little as necessary with customary habits of eating. . . ."

*From the Metabolic and Nutrition Division, Department of Medicine, Queens General Hospital.

Received for publication, November 28, 1938.

The solution of zinc insulin crystals used in this study was generously supplied by Frederick Stearns & Co., of Detroit. This material was formerly known as crystalline insulin.

Lastly, in those diabetics whose urines *were* kept consistently sugar free the problem of night hypoglycemia often arose. Even an intelligent experienced diabetic has difficulty in combating protamine insulin reactions of notoriously insidious onset and prolonged hypoglycemic effect.

Since protamine zinc insulin did not solve all the problems encountered in the use of standard insulin, it was often felt that an insulin whose action was intermediate between these two might make our armamentarium for treating the diabetic more complete.

Crystalline insulin, first isolated by Abel of Johns Hopkins, was prepared for the first time in 1935 in amounts large enough for clinical investigation by Melville Sayhun of the Stearns Laboratories. In 1936 Freund and Adler[5] demonstrated that crystalline insulin had an onset of action more rapid than that of protamine insulin but was effective two or three times longer than standard insulin. The number of injections and units required were often less than for standard insulin. Mains and McMullen[6] and Altshuler and Leiser[7] came to similar conclusions. Wilder[4] also reported the action of crystalline insulin to be intermediate to the other two insulins. Barach,[8] finding this insulin to be an improvement over standard insulin in 12 out of 21 cases, noted that reactions occurred only one-fourth as frequently as with standard insulin.

Why the crystalline insulin has a prolonged action has not yet been definitely determined. Experiments have shown that the amount of zinc in the crystalline insulin solution is not a factor.[9] Sayhun[10] believes that the relative insolubility of the crystals at the tissue pH is responsible.

In February, 1938, we began to observe the action of crystalline insulin, now known as solution of zinc insulin crystals, in diabetic patients. For certain difficult cases, an insulin which would prevent extreme and rapid oscillations between hyperglycemia and hypoglycemia, or which would not permit prolonged hyperglycemia followed by prolonged hypoglycemia, might prove useful. Reduction in the number of doses of insulin below the customary number of standard insulin injections or reduction in the total daily amount of insulin needed were only secondary considerations. Primarily, we were interested in finding something which would keep the diabetic patient's blood sugar within physiologic ranges throughout the entire day.

Uncomplicated hospitalized diabetics, ranging in age from 12 to 60 years, were chosen. Older patients were not selected because they usually have mild diabetes, take little insulin, and the question of fluctuations is not a prominent factor.

As a preliminary study, we compared the actions of standard insulin, protamine zinc insulin, and zinc insulin crystals, respectively, in three apparently normal individuals. These subjects were each given diets of 250 gm. carbohydrates, 80 gm. protein, and 130 gm. fat, divided into three equal meals. Each individual's twenty-four-hour blood sugar curve was first estimated on diet alone. We then gave single doses of 10 units of standard insulin, zinc insulin crystals, and protamine zinc insulin, respectively, for one day each. Four days were allowed to elapse between each series of blood sugar determinations in order to obviate any possible effect of the previous dose of insulin. In all three subjects it was evident as already established that the maximum effect of standard insulin

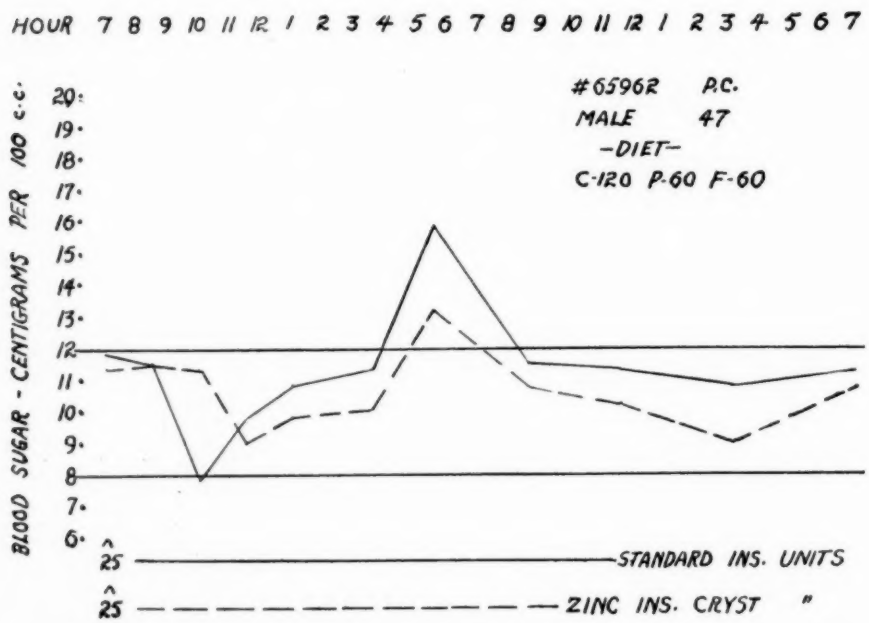

Fig. 1.—On one dose of standard insulin sharp effect of insulin at 10 A.M., with high peak at 5:30 P.M. On same dose of zinc insulin crystals, tendency to follow blood sugar curve as with standard insulin. However, only slight peak at 5:30 P.M., with generally more even stabilization.

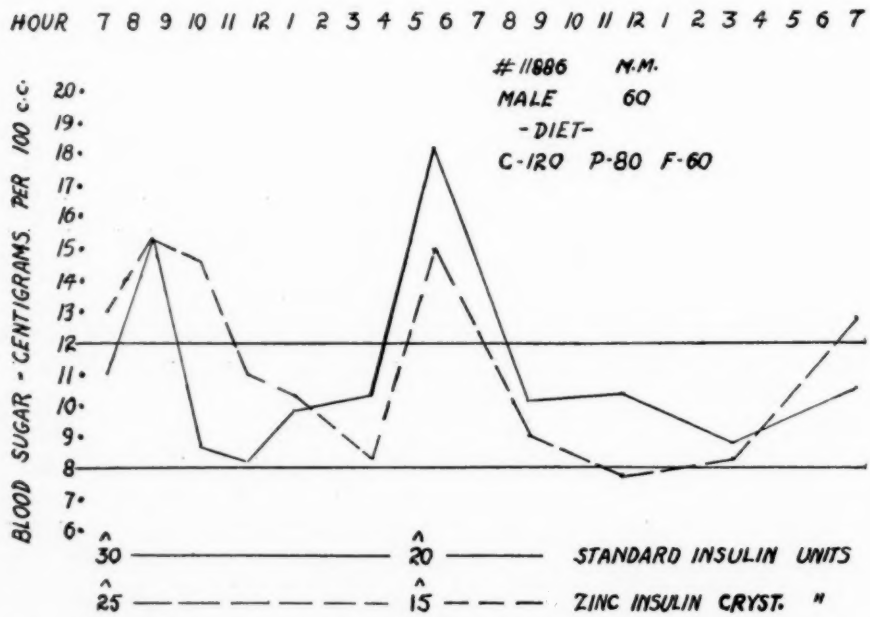

Fig. 2.—With two doses of standard insulin, fluctuations evident. On two doses of zinc insulin crystals with reduction of total dosage by ten units, an improved curve. A postprandial fall instead of rise at 1 P.M. with zinc insulin crystals. Note how crystalline curve otherwise follows standard curve.

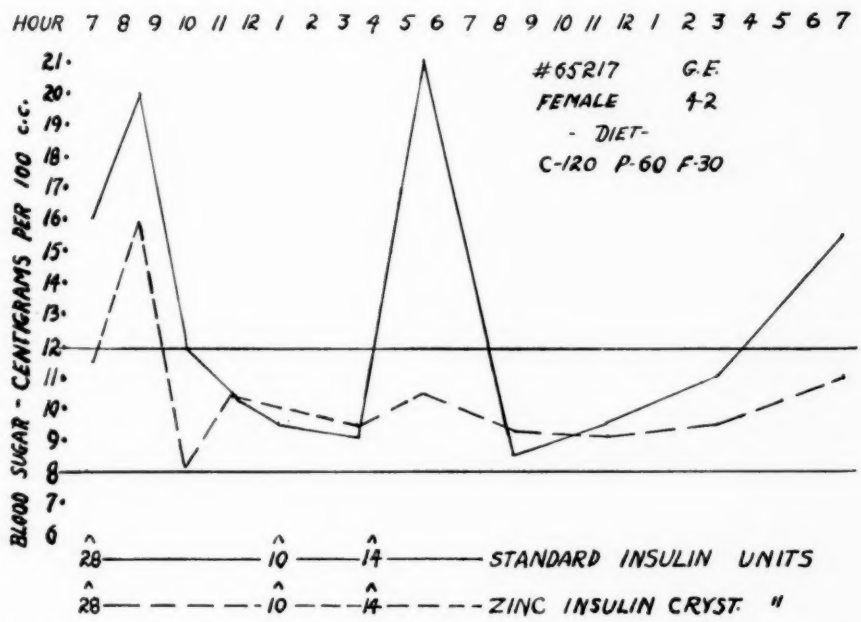

Fig. 3.—On three doses of standard insulin, wide fluctuations. On equal doses of zinc insulin crystals marked leveling off with uniformly stable blood curve.

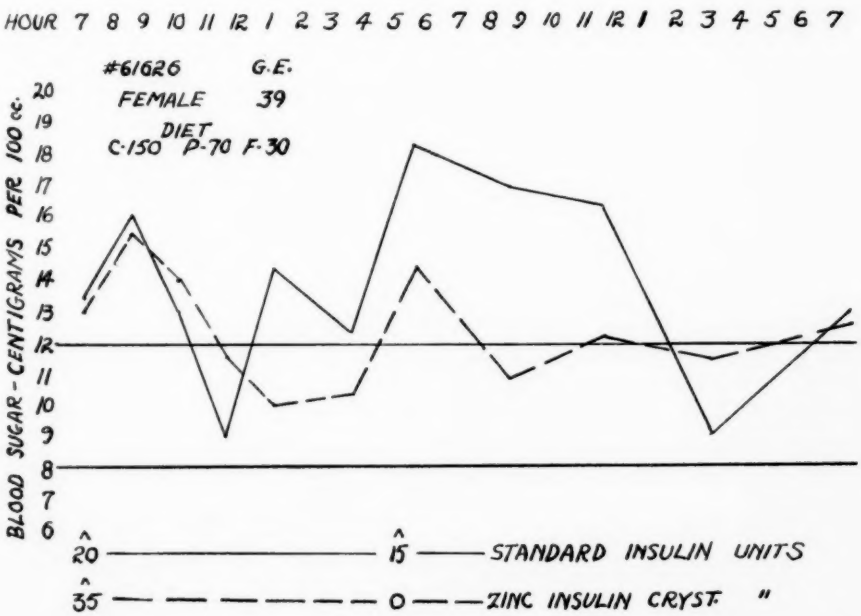

Fig. 4.—On two doses of standard insulin markedly irregular curve, although sugar free and ordinarily considered good control. On one dose of zinc insulin crystals equal to the sum of the two doses of standard, a very marked improvement in blood curve and evident physiologic control.

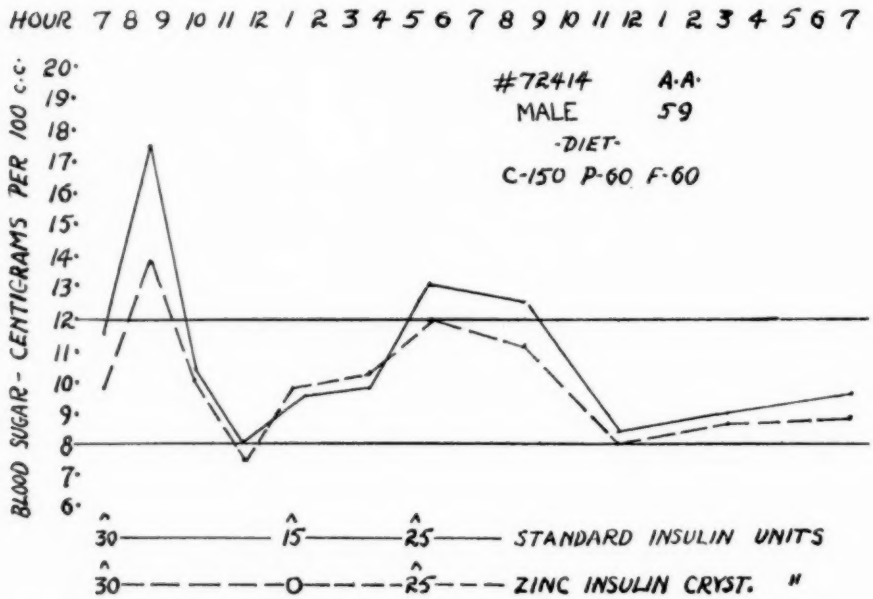
HOUR 7 8 9 10 11 12 1 2 3 4 5 6 7 8 9 10 11 12 1 2 3 4 5 6 7
BLOOD SUGAR - CENTIGRAMS PER 100 c.c.
#72414 A.A.
MALE 59
-DIET-
C-150 P-60 F-60
^30 ^15 ^25 STANDARD INSULIN UNITS
^30 O ^25 ZINC INSULIN CRYST. "

Fig. 5.—On three doses of standard insulin ordinarily good control. With two doses of zinc insulin crystals and the elimination of one dose of 15 units, a markedly improved blood curve indicating excellent control.

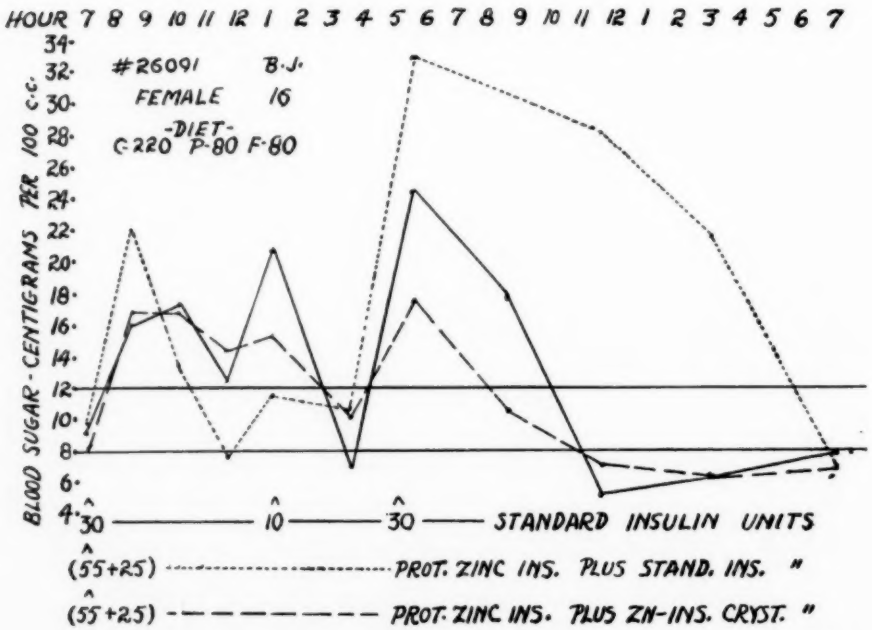
HOUR 7 8 9 10 11 12 1 2 3 4 5 6 7 8 9 10 11 12 1 2 3 4 5 6 7
BLOOD SUGAR - CENTIGRAMS PER 100 c.c.
#26091 B.J.
FEMALE 16
-DIET-
C-220 P-80 F-80
^30 ^10 ^30 STANDARD INSULIN UNITS
(^55+25) PROT. ZINC INS. PLUS STAND. INS. "
(^55+25) PROT. ZINC INS. PLUS ZN-INS. CRYST. "

Fig. 6.—Poor regulation with three doses of standard insulin. No improvement with protamine zinc insulin and standard insulin as a supplement. A very much improved curve with protamine zinc and zinc insulin crystals.

was four hours in length, that of protamine zinc insulin for twenty-four or more hours, and that of zinc insulin crystals for fourteen hours.

In diabetic patients, we feel that, as Sindoni[11] has emphasized, the frequently sharp and high postprandial rises are important. A test of the zinc insulin crystals would be whether or not these particular oscillations were affected. In addition the interval between 7 at night and 7 in the morning should receive more consideration. Unless blood sugars are determined with reasonable frequency, this period may be deceptive. For these reasons the series of blood sugars determined on each patient included samples one and one-half hours after meals and several determinations throughout the night. Blood for sugar determination by the modified Folin micromethod was taken at 7, 8:30, 10, and 11:30 A.M. and 1, 3, 5:30, 8:30, and 11:30 P.M., and at 3 and 7 o'clock the succeeding morning. These samples were all taken by two of us and all colorimetric determinations were made by one of us.

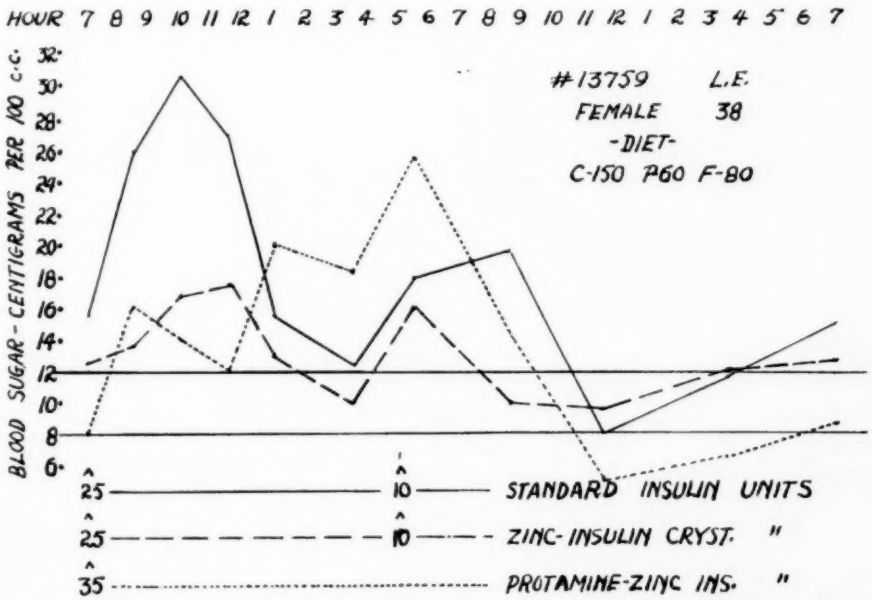

Fig. 7.—Regulation with standard insulin poor. One dose of protamine zinc insulin equivalent to the total of standard did not improve the blood curve. On two doses of zinc insulin crystals equivalent to the standard insulin, a physiologic blood curve resulted. The patient felt "entirely normal" on this regulation for the first time in her experience with insulin.

Because of hospital routine, the diets were served at 7:15 A.M., 11 A.M., and 4 P.M., having been divided into three equal meals unless protamine zinc insulin was being used. In such instances the carbohydrate was divided as follows: one-sixth for breakfast, one-third each for lunch and supper, and one-sixth at about 8:30 P.M.

In many patients blood sugar curves were repeated without changing the type or dose of insulin in order to rule out the question of time under treatment as a factor in improved curves. Patients showing variable blood sugar curves on fixed doses of insulin were excluded from this study. In all, a total of 1,866 blood sugar determinations were performed. Figs. 1 to 8 are examples of various methods used to establish best regulation.

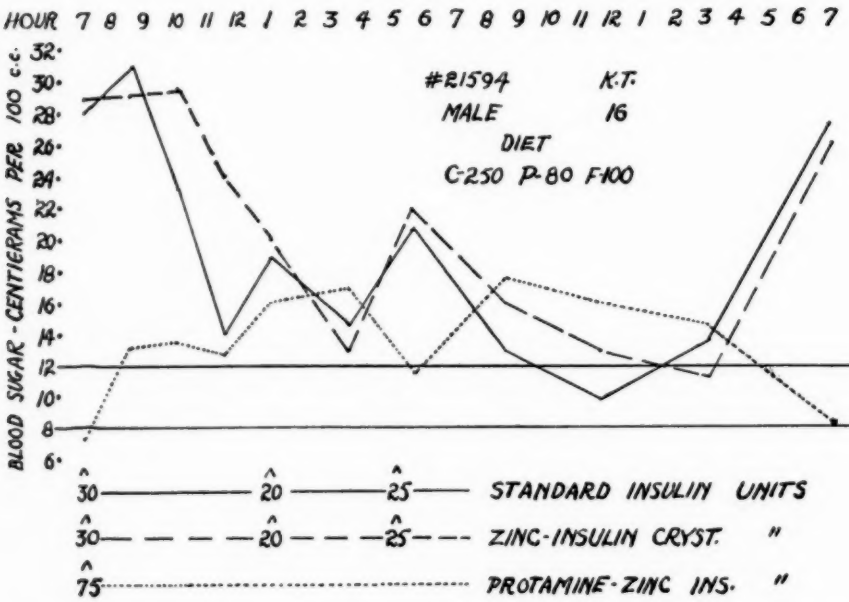

Fig. 8.—Poor control with three doses of standard insulin and equivalent dosage of zinc insulin crystals. On one dose of protamine zinc insulin equal to the total of three doses of the other, very good control with relatively good blood curve. Note how crystalline curve follows standard insulin curve. This is the only patient in our series in whom one dose of protamine zinc insulin alone produced relatively good control.

ANALYSIS OF RESULTS

The total number of patients in this series was 35. This excluded those diabetics whose blood sugar curves were unstable on fixed doses of insulin for various reasons.*

Total number of patients in whom SI† and ZIC† were compared			35
Effect of ZIC equal to or better than SI in			35
ZIC better than SI in		23	
ZIC better than SI in equal amount and number of injections in	10		
Better control on ZIC with amount reduced and injections reduced from three to two in	6		
Better control on ZIC with amount reduced and injections reduced from two to one in	4		
Better control on ZIC with amount unchanged but injections reduced from two to one in	3		
SI equal to ZIC in		12	
Number of patients who received PZI,† ZIC and SI separately, as well as combinations of PZI plus ZIC and PZI plus SI			16
Best control on ZIC alone		12	
Best control on PZI alone		1‡	
Best control on PZI plus ZIC		3	

COMMENT

In a study of the action of zinc insulin crystals on 35 hospitalized uncomplicated diabetic patients we found, as already shown, that crystalline insulin has

*The lowest dose of insulin to any case was 25 units per day. The highest was 85 per day.

†Abbreviations: SI for standard insulin, ZIC for zinc insulin crystals, and PZI for protamine zinc insulin.

‡Curiously enough, this patient, a male aged 17, could not be regulated at all on either zinc insulin crystals or standard insulin.

a duration of action intermediate between that of standard insulin and protamine zinc insulin. This action was effective in most instances from eight to sixteen hours.

In nearly all patients the amplitude of blood sugar fluctuations observed with standard insulin regulation was decreased when crystalline insulin was substituted. This was most noticeable in those patients who presented very marked fluctuations.

In all patients substitution of an equal amount of zinc insulin crystals for standard insulin produced improvement in the blood sugar graphs. When the dose of zinc insulin crystals was reduced, some patients still revealed some lowering of the blood sugar curve. In no case did standard insulin produce a more satisfactory result than did zinc insulin crystals.

Most of the patients voluntarily stated that they felt much better on zinc insulin crystals than on standard insulin. We believe that this is due to the sharper fluctuations produced by standard insulin. Patients with blood sugar levels of 50 mg. rarely showed untoward symptoms when zinc insulin crystals were used, whereas there was definite shock when the same level was produced by standard insulin.

Where it was necessary to supplement protamine zinc insulin, substitution of zinc insulin crystals for the standard insulin supplement usually produced a definite improvement in the blood sugar curve, assuring the patient of a more stable regulation.

CONCLUSION

Solution of zinc insulin crystals is a valuable adjunct to the treatment of diabetes mellitus. Its action is much more prolonged and steadier than that of standard insulin, but is shorter in duration than that of protamine zinc insulin. It conveniently assumes the role of an intermediate between the latter two. In regulation it does what standard insulin does but apparently produces a less fluctuant type of blood sugar curve. As yet we have not familiarized ourselves with its action in diabetics with acidosis and so cannot comment on the efficiency of zinc insulin crystals in such situations.

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CHANGES IN SPLEEN SIZE, BLOOD PRESSURE, AND ERYTHROCYTE COUNT AFTER THE ADMINISTRATION OF BENZEDRINE SULFATE (PHENYL-1, AMINO-2 PROPANE SULFATE) IN DOGS*

J. L. Pinkston, B.A., and J. O. Pinkston, Ph.D.
Beirut, Lebanon

THE response of the spleen to certain of the sympathomimetic amines has been observed by many investigators. Contraction of the spleen in response to epinephrine has been demonstrated by Hoskins and Gunning,[1] Tournade and Chabrol,[2] Barcroft,[3] and others. Izquierdo and Cannon[4] carried out a study on emotional polycythemia; they consider that splenic contraction may be responsible for an increase of up to about 20 per cent in the number of circulating erythrocytes, and that other factors enter into any greater increase.

The response of the spleen of the dog to ephedrine was briefly studied in 1930 by Marcou and Barcroft (see Barcroft[3]). They observed marked contraction, which passed off gradually during a period of about two hours. Davis,[5] using the technique devised by Barcroft and Stephens,[6] also showed contraction of the exteriorized spleen in response to ephedrine in dogs. Simpson and Cadness[7] found the increase in erythrocyte, leucocyte, and platelet counts in response to ephedrine essentially the same before and after splenectomy in the guinea pig, and they conclude: "The spleen is therefore not responsible for these changes in this animal."

Detrick, Millikan, Modern and Thienes,[8] in a study of the effects of benzedrine (amphetamine) on isolated smooth muscles, state in their summary: "In high concentrations (10^{-4} or higher) benzedrine caused contractions of all smooth muscles."

In a study of the functional and tissue changes following large doses of benzedrine sulfate (amphetamine sulfate) in the albino rat, Ehrich and Krumbhaar[9] found at autopsy that animals which died after having received 150 to 400 mg. of the drug showed contraction of the spleen; they attributed this contraction to the action of the benzedrine. These authors also found that in general an erythrocytosis occurred following benzedrine administration.

Myerson, Loman, and Dameshek[10] say concerning certain responses to benzedrine in man: "Its effects on the blood cellular constituents are probably due to vasoconstriction of the spleen and other reservoirs of blood cells resulting in an increased number of cells in the peripheral blood." Schube, Raskin, and Campbell[11] found no demonstrable permanent alteration of the cellular constituents or hemoglobin of the blood in human beings following the administration of benzedrine in dosage within the clinical range.

*From the Department of Pharmacology, School of Medicine, American University of Beirut, Beirut, Lebanon. Aided by a grant from Smith, Kline and French Laboratories.
Received for publication, December 7, 1938.

The present experiments were undertaken in order to investigate more fully the response of the spleen to the intravenous administration of benzedrine, and, in particular, to seek any correlation between splenic response and erythrocytosis occurring under the same circumstances.

Since the spleen of the dog contains sufficient smooth muscle to show visible changes in size when contraction occurs, photographs taken at stated intervals were used to record these changes. The position and adjustment of the camera remained constant throughout each experiment. This method of recording has obvious limitations, but serves to show gross alterations in size.

The experiments fall into two general groups: one in which we have sought to discover the possible correlation between changes in blood pressure and changes in spleen size, and another in which any correlation between changes in spleen size and erythrocyte count has been sought.

I. RESPONSES OF THE SPLEEN AND THE BLOOD PRESSURE

Methods.—A series of healthy male and female dogs was used, the average weight of dog being about 9 kg. They were anesthetized in respective cases with ether and chloralose, dial,* and nembutal (the responses were essentially the same irrespective of the anesthetic used). Body temperature was maintained within the normal range by means of a heating pad, and checked by a rectal thermometer kept in place throughout each experiment. The spleen was delivered through a left lateral incision with as little handling as possible, and then surrounded by towels kept moist with warm physiologic saline. The splenic blood supply was carefully left unimpeded, and the organ was exposed only while being photographed. Very little blood was lost during the operative procedures. Blood pressure was recorded from the carotid artery by means of an ordinary mercury manometer. In all cases each injection of benzedrine consisted of 1 mg. per kg., a dosage chosen arbitrarily on the basis of its effectiveness and nontoxicity in the acute procedure.

Procedures of three types were carried out:

(a) Experiments (13) in which a first injection of benzedrine was given immediately after exposure of the spleen, and followed within one to two hours by a second injection.

(b) Experiments (5) in which a single injection was given immediately after exposure, and the effects followed for six to eight hours.

(c) Experiments (7) in which the spleen was exposed as described for six to eight hours, and an injection of the drug given only after this period of exposure.

Results.—(a) The first injection was followed within a few seconds by a rise in blood pressure of from 21 to 91 per cent (average, 48 per cent), which reached its height within about one and one-half minutes, and had returned to approximately its initial level within a maximum of fifteen minutes. Contraction of the spleen began while the pressor response was still fairly marked, and reached a maximum within about thirty minutes, long past the time when the pressor response had subsided. The second injection, given within one to two hours

*Supplied by the Ciba Laboratories, Lyon, France.

(before there was perceptible relaxation of the spleen), was followed within a few minutes by a visibly greater contraction (Fig. 1, B and C). In a few cases there was a slight rise in blood pressure following the second injection, but the majority of cases showed either no change or a moderate fall of the blood pressure, which then gradually returned to the original level. This alteration in the blood pressure response to successive injections of benzedrine in the same animal has been observed by Tainter,[13] Detrick, Millikan, Modern, and Thienes,[8] and others.

The blood pressure level immediately before injection varied from 90 to 180, without appreciably affecting the nature or degree of response to the drug.

(b) Following a single injection immediately after exposure, the splenic contraction was observed to persist with but slight gradual relaxation for up to six hours (Fig. 2). The blood pressure response was as in (a).

(c) In the spleen exposed for six hours, slight gradual contraction occurred (Fig. 3, A, B, and C) without significant change in the blood pressure level during that period.* At the end of six hours' exposure, an injection was followed within a few minutes by visible contraction (Fig. 3, D) and a pressor response of the type described in (a).

II. RESPONSES OF THE SPLEEN AND THE ERYTHROCYTE COUNT

Methods.—A second, similar group of dogs was used. Dial anesthesia was employed throughout, and a single intravenous injection of warm physiologic saline was given soon after anesthetization. The amount, 25 to 75 c.c., was determined by the size of the animal, and at least twenty minutes was allowed to elapse before beginning blood counts.

Erythrocyte counts were made on venous blood at recorded intervals. Samples were obtained from alternate leg veins by means of a perfectly dry 2 c.c. syringe and a 25 gauge needle; care was taken that the limbs were warm and not constricted. About 0.5 c.c. of blood was withdrawn, placed on a paraffin-covered watch glass, and immediately taken up into a Trenner pipette for dilution with Hayem's fluid. Counts were made from a Neubauer chamber with a standardized cover slip; the same pipette and chamber were used throughout the series, and conditions and technique kept as uniform as possible. A sufficient number of counts was done in each case to check closely on technical accuracy.

In the cases indicated, the spleen was removed, and the abdomen was closed after complete hemostasis had been achieved.

Changes in spleen size were recorded as in I, but blood pressure was not recorded. Each injection of benzedrine consisted of 1 mg. per kg.

Procedures of three types were employed:

(a) Experiments (10) in which a series of counts were carried out in the anesthetized animal, and the spleen then exposed as described. After a second group of counts, the benzedrine was administered, and the erythrocyte count followed for at least one hour thereafter.

*That the exposed spleen will shrink somewhat, and that after contraction it will not return to quite its original size while exposed, is to be expected, as has been demonstrated by Barcroft and his co-workers.

(b) Experiments (6) in which the same procedure was carried out, except that the spleen was removed rather than simply exposed.

(c) Experiments (6) in which the adrenals were removed* in a preliminary acute operation, and a group of counts were then taken. After establishment of the erythrocyte level, benzedrine was administered, and the count followed as in (a) and (b).

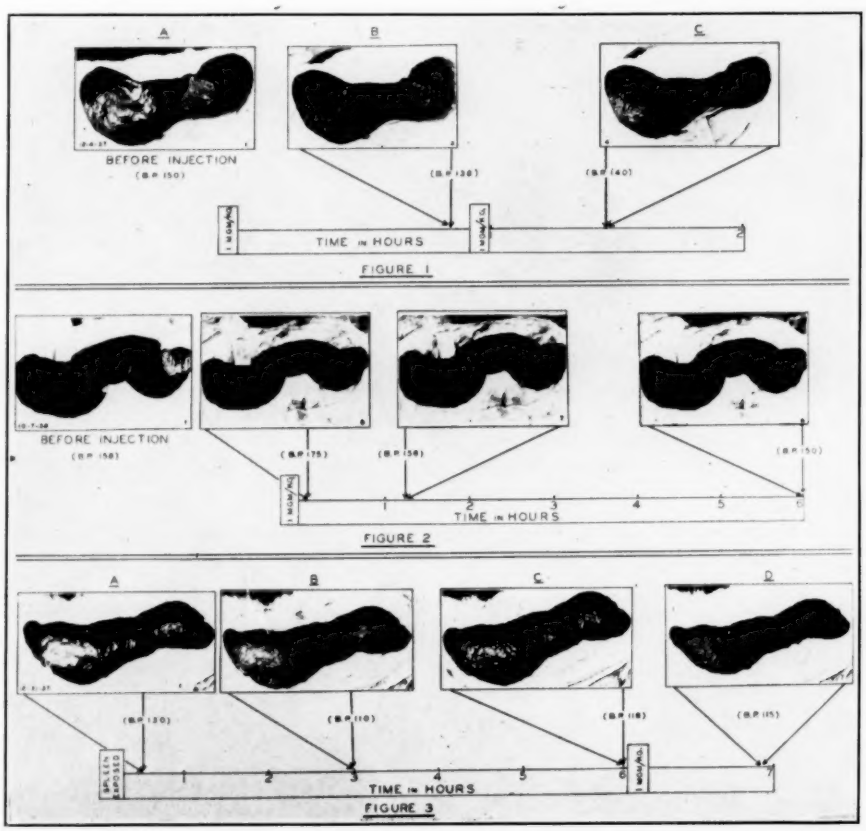

Fig. 1.—Changes of spleen size in the dog after the intravenous administration of two doses of 1 mg. per kg. of benzedrine; one given within ten minutes after exposure of the spleen, the second within one hour after the first.

Fig. 2.—Changes of spleen size in the dog after the intravenous administration of a single 1 mg. per kg. dose of benzedrine within ten minutes after exposure of the spleen.

Fig. 3.—*A*, *B*, and *C*. Changes of spleen size in the dog during six hours of simple exposure. *D*. Change following a single dose of 1 mg. per kg. of benzedrine given after six hours of exposure.

Results.—(a) The erythrocyte level was established by means of three or more counts before the administration of benzedrine in each case. Following the injection, there occurred a rise of from 2 to 29 per cent within twenty to seventy minutes, with an average rise of 16 per cent within an average time of thirty-six minutes. The extreme figures of a 2 per cent rise in seventy minutes were obtained in a single experiment (8/23/38, Fig. 5); the next lowest rise was 9 per cent, and the next longest time forty-five minutes.

*In one dog (11/2/38), Fig. 7, the adrenals were tied off. Autopsy was done at the conclusion of the experiment in order to be certain that the adrenals had been excluded from the circulation.

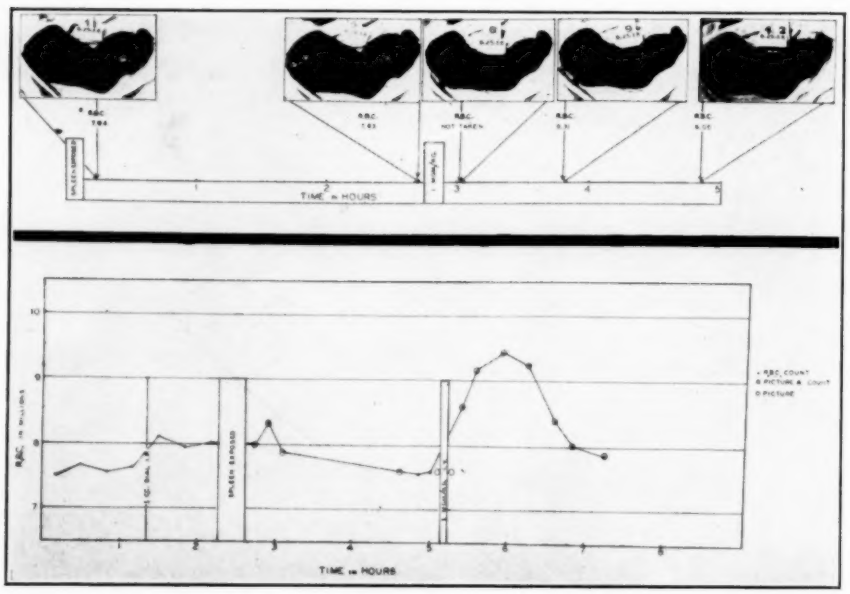

Fig. 4.—Changes of spleen size and erythrocyte count in the dog after exposure of the spleen and subsequent intravenous administration of 1 mg. per kg. of benzedrine.

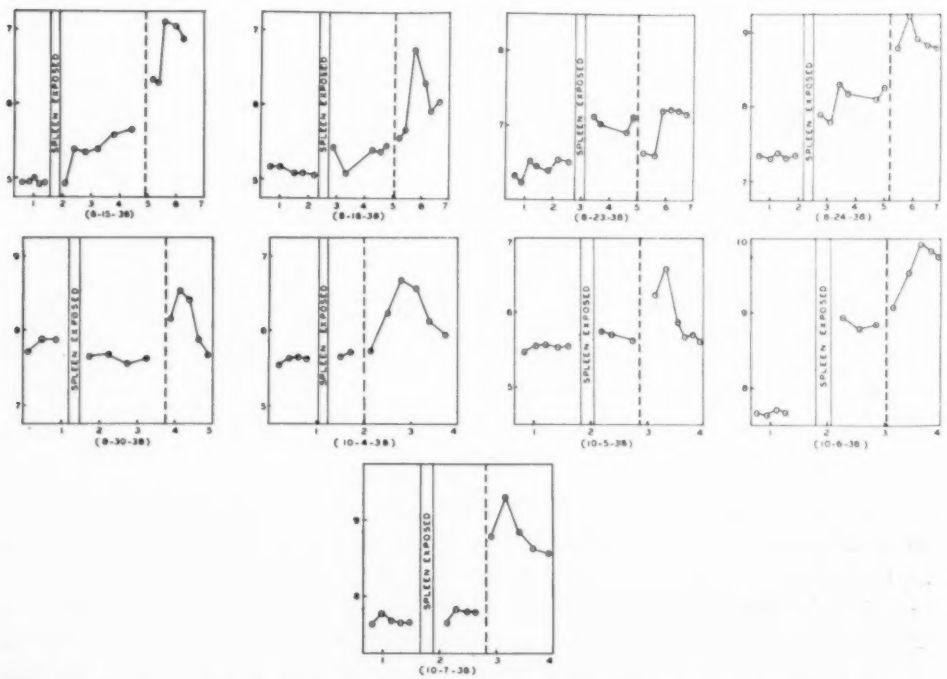

Fig. 5.—Changes of erythrocyte count in 9 dogs after exposure of the spleen and subsequent intravenous injection of 1 mg. per kg. of benzedrine (dotted line). Abscissae (scale value not the same in every case), time in hours; ordinates, erythrocyte count in millions.

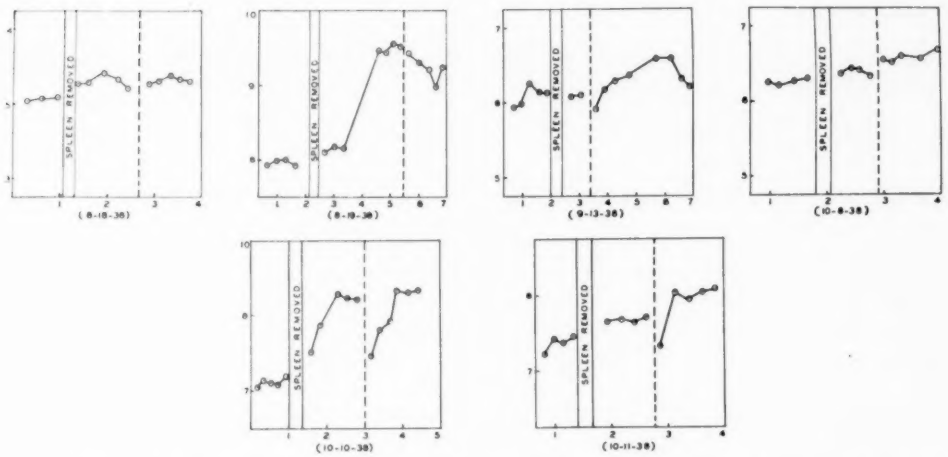

Fig. 6.—Changes of erythrocyte count in 6 dogs after removal of the spleen and subsequent intravenous injection of 1 mg. per kg. of benzedrine (dotted line). Abscissae (scale value not the same in every case), time in hours; ordinates, erythrocyte count in millions.

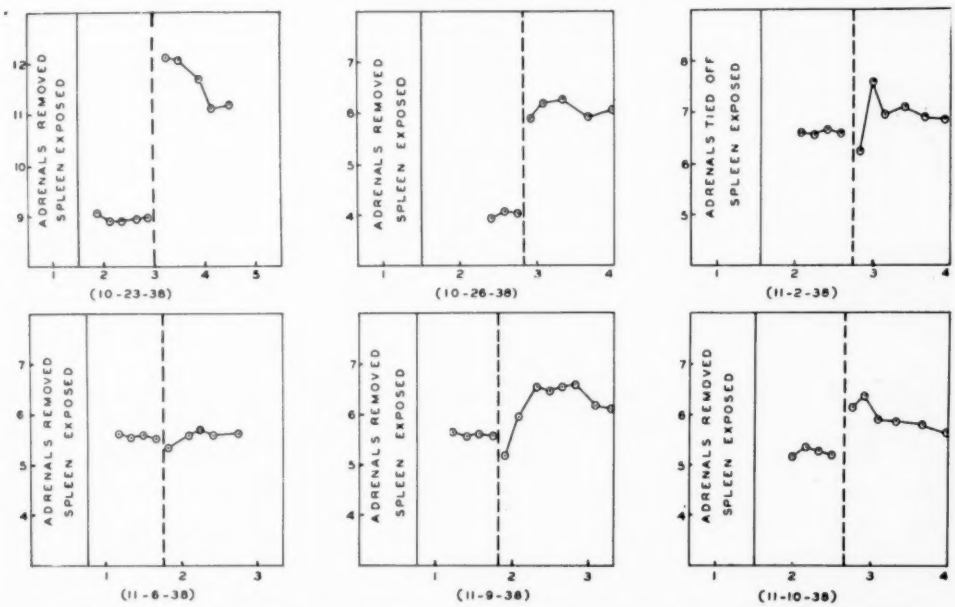

Fig. 7.—Changes of erythrocyte count in 6 dogs after removal of the adrenals, with exposure of the spleen and subsequent intravenous injection of 1 mg. per kg. of benzedrine (dotted line). Abscissae (scale value not the same in every case), time in hours; ordinates, erythrocyte count in millions.

A typical experiment of this type is illustrated in Fig. 4, and the remainder of the group is represented graphically in Fig. 5.

(b) In those animals that underwent splenectomy, benzedrine administration was followed in some cases by an immediate, slight fall and then a very gradual, moderate rise of the blood count. In other cases, this rise occurred slowly without the preliminary fall. In no case did the rise of red blood cell count exceed 7 per cent in the period of from sixty to one hundred and eighty minutes during which the counts were followed after injection. A single case

showed a fall of 5 per cent within seventy minutes, and the count remained at 2 per cent below the initial level at the end of ninety minutes, when counts were discontinued.

This group is represented graphically in Fig. 6.

(c) In those cases in which the adrenals were removed (or tied off, as in one case), rises in erythrocyte counts were observed following the administration of benzedrine. The results were so variable, ranging from 2 to 55 per cent change within ten to sixty minutes (Fig. 7), that no exact conclusion concerning the role of the adrenals can be reached from this short series. It is clear, however, that the erythrocytosis observed after administration of benzedrine occurs in the adrenalectomized animal.

DISCUSSION AND CONCLUSIONS

The role of the spleen in the occurrence of erythrocytosis in dogs and cats following the administration of certain of the sympathomimetic amines and after sympathetic stimulation has been well established by investigators previously cited in this paper. In the present experiments, benzedrine sulfate was consistently found to cause contraction of the spleen, accompanied by promptly occurring erythrocytosis, after intravenous administration of 1 mg. per kg. in the dog. This erythrocytosis was likewise observed along with splenic contraction in adrenalectomized dogs, but did not occur in splenectomized dogs having intact adrenals.

These results, along with the extensive investigations of other workers, indicate that the spleen is the principal factor responsible for prompt and significant increases in the red blood cell count in the dog.

SUMMARY

1. In anesthetized dogs having the spleen exposed acutely, prolonged contraction of the spleen and a definite, transitory rise of blood pressure were observed following the intravenous administration of 1 mg. per kg. of benzedrine. A second injection of 1 mg. per kg. in the same animal was followed by further splenic contraction, but blood pressure showed either no change or a slight, transitory fall (13 experiments).

2. In anesthetized dogs having the spleen exposed acutely, contraction of the spleen following the intravenous administration of 1 mg. per kg. of benzedrine was observed to be accompanied by a rapidly occurring erythrocytosis of significant degree (10 experiments).

3. In anesthetized dogs having the spleen exposed and the adrenals removed acutely, responses of the spleen and the erythrocyte count after benzedrine administration were essentially the same as in 1 and 2 (6 experiments).

4. In anesthetized dogs having the spleen removed acutely, and the adrenals intact, no significant erythrocytosis occurred following the administration of benzedrine, except after a period of at least an hour, and probably not in direct response to the action of this drug (6 experiments).

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CHYLOTHORAX AND CHYLOUS ASCITES*

Report of a Case With Lipid Analyses

George T. Harrell, M.D., Dana M. Street, M.D., and Raymond Reiser, Ph.D., Durham, N. C.

THE accumulation of chylous fluid in the serous cavities of the body is an event of relatively rare occurrence. Bauersfeld[1] noted 49 cases of traumatic chylothorax reported in the literature. Yater[7] found less than 100 cases of non-traumatic chylothorax, and only 3 of chylopericardium. Chylous ascites may accompany chylothorax, or it may occur alone. The commoner causes of chylous effusion are: trauma, invasion, compression, or occlusion of the thoracic duct by a malignant tumor or leucemia, thrombosis of the great veins at the exit of the duct, or infection, usually tuberculous, with obstruction of the duct and smaller lymphatics.

Lee[4] has experimentally shown that chylous fluid rarely forms from occlusion of the duct alone. The extent of the lymphatic supply, the patency of collaterals,

*From the Departments of Medicine and Pathology, Duke University School of Medicine, Durham.

Received for publication, December 30, 1938.

the level and completeness of the obstruction are other factors. Attempts have been made to classify the fluids as chylous, chyliform, or pseudochylous by their appearance and fat content.[3] These groups are not distinct, for the appearance and composition are dependent on the size of the fat particles, the relative proportion of lymph and chyle, the cellular content, the diet, and the lipid partition. Lactescent fluids are also said to occur from degeneration of the cells in exudates and from dispersion of such substances as "glycoproteid" or lecithin-globulin complex.

The reports in the earlier literature of the chemical composition of these fluids have been reviewed by Gandin.[3] Since the introduction of more modern and accurate methods of fat analysis, determinations on human material have been rare. Robinson and his co-workers[6] have analyzed fluids experimentally produced in cats and dogs. Since none of the previous reports have compared the fluid in the various cavities, it was thought the studies upon the following case warranted recording.

CASE REPORT

A 49-year-old white tenant farmer was first admitted January 31, 1937, complaining of the loss of 45 pounds in weight in the preceding three months. For five years the patient had noted some burning in the epigastrium with fullness after meals. For three months he had been vomiting shortly after meals once or twice daily. He had taken little by mouth for six days.

On admission the temperature was 36.5° C., pulse 100, respirations 20, and blood pressure 135/85. The patient was weak, chronically ill, and showed evidence of weight loss. A smooth, firm, nontender mass was present in the epigastrium, and extended 4 cm. below the xiphoid process. The remainder of the physical examination revealed no abnormal findings. Wassermann and Kahn tests were negative. The blood sugar on admission was 880 mg. per cent; chlorides, 320 mg. per cent; carbon dioxide combining power, 96 volumes per cent; nonprotein nitrogen, 125 mg. per cent; cholesterol, 128 mg. per cent. Stools were negative for occult blood. X-rays of the gastrointestinal tract showed only a high hypertonic stomach with thickened rugae.

After an afebrile course of nineteen days on a diabetic regime, he was discharged symptom free. During the next two months his insulin requirement steadily reduced.

He was re-admitted on April 24, 1937, three months after his first admission, complaining of an upper respiratory infection of eighteen days' duration, associated with abdominal pain and vomiting. Five days previously he had noticed a swelling in the left side of his neck. At this time the temperature was 36.5° C., pulse 140, respirations 22, blood pressure 114/80. The positive physical findings were limited to small palpable lymph nodes in the neck, an indefinite mass in the region of the left sternocleidomastoid muscle, moist râles at the lung bases, and absent knee jerks. No mass was palpable in the epigastrium, and there was no jaundice.

The red blood cell count was 3,800,000, the white blood cells totaled 16,600, with 96 per cent polymorphonuclear leucocytes. The blood chemistry findings were within normal limits. Gastrointestinal x-rays demonstrated a triangular notch on the greater curvature of the stomach, and delay in emptying of the duodenum with the second and third portions fixed and rigid. This was interpreted as extrinsic involvement by tumor. On admission chest plates revealed prominent hilar shadows, but no fluid. Metastatic carcinoma was found in microscopic sections of a lymph node removed from the neck.

Fluid rapidly accumulated in both sides of the chest and in the abdomen. New tumor nodules appeared in the left side of the neck, with edema of the right side of the face and the left hand. The mucous membranes became cyanotic. He died quietly on his eighteenth day in the hospital.

Autopsy.—Autopsy was performed thirty minutes after death. Externally the cervical and left axillary lymph nodes were enlarged. Two and eight-tenths liters of grayish yellow chylous fluid, with an odor resembling that of milk, were removed from the peritoneal cavity, and one liter of similar fluid from each of the pleural cavities. The pericardium contained 20 c.c. of clear straw-colored fluid. The peritoneal surfaces were smooth, glistening, and free, except for old fibrous adhesions between the gall bladder, duodenum, and transverse colon. The lymphatics in the serosa of the entire intestine were distended with chyle, resembling the lacteals of experimental animals fed on fat (Fig. 1).

The hilar lymph nodes were greatly enlarged, up to 3 cm. in diameter, and contained pockets of creamy material, 3 mm. in size, which could be expressed from the sinuses. A gray tumor nodule, 1 by 0.5 cm. in size, was found in a large vein of the posterior portion of the right lung. No tumor nodules were found in the lung parenchyma or bronchi.

Fig. 1.—Loop of small intestine showing lacteals filled with chyle.

The liver weighed 1940 gm.; small, round, firm, white nodules, 2 mm. in size, were scattered throughout. No cirrhosis was present. The bile ducts and portal vein at the hilum of the liver were surrounded by a 2 cm. mass of tumor tissue, but the ducts were not obstructed. The pancreas, yellowish brown in color, weighed 160 gm. and was densely embedded in fibrous tissue. The head was enlarged, surrounded by nodules of tumor tissue, and contained a single small nodule 3 cm. in size. The soft spleen weighed 150 gm.; the splenic vein contained tumor tissue. The adrenals weighed 40 gm. together; the tissue was almost entirely replaced by tumor nodules of 1 cm. size or less.

On the greater curvature of the stomach was an old ulcer, perforation of which had been closed by adhesion between the stomach wall, the lower margin of the pancreas, and the posterior abdominal wall. The walls of the second and third portions of the duodenum were thickened and surrounded by dense fibrous tissue; the lumen was narrowed.

The thoracic duct was enlarged and patent to a point above the arch of the aorta, where it was buried in a mass of supraclavicular nodes, 6 cm. in diameter. No lymphatic collaterals, right thoracic, or accessory ducts were seen, though dye was injected into the thoracic duct. No thrombus was noted in the great veins of the neck or thorax.

In microscopic preparations of the ulcer in the stomach large, eosinophilic, vacuolated cells, with eccentrically placed nuclei, were found at the edges, infiltrating the wall, suggest-

ing the origin of the tumor from mucous cells. No glandular patterns or areas of colloid were found. At numerous other places throughout the body, the tumor cells were anaplastic and mitoses were numerous. Dilated lymphatics, filled with eosinophilic material staining brightly with scharlach R and containing strands and sheets of tumor cells, were found in the mucosa of the duodenum and gall bladder, fibrous tissue septa of the pancreas, and other viscera. In lymph nodes, the tumor showed necrosis. The branches of the thoracic duct, which was multiple near its termination, were almost filled with tumor cells (Fig. 2). Although the primary site was probably in the stomach, the pancreas, gall bladder, and duodenum could not be definitely excluded.

Analysis of Fluids.—The characteristics of the fluids aspirated are given in Table I. All were sterile on culture and contained small numbers of large oval cells, twice the size of lymphocytes, which were thought to be tumor cells. Fat droplets of varying size and number were stained by sudan III.

TABLE I

	RIGHT PLEURA		LEFT PLEURA	PERITONEUM
Date	5/1/37	5/5/37	5/5/37	5/3/38
Quantity	450 c.c.	--------	130 c.c.	4 liters
Character	opalescent	opalescent	turbid	creamy
Color	yellow	greenish yellow	chylous	--------
Specific gravity	1.016	--------	--------	1.022
W.B.C.	125/mm³	450/mm³	315/mm³	400/mm³
R.B.C.	75/mm³	280/mm³	90/mm³	100/3
Protein*	3.5 gm. %	--------	--------	4.0 gm. %

*Esbach tube method.

The lipid analyses were done by a modification of the method previously reported by one of us.[5] The phospholipids were determined according to Boyd[2] and the free cholesterol on the acetone washings. The bound cholesterol digitonide was determined on the washings from the free cholesterol and the neutral fat fatty acids on the washings from the bound. The results on fluid aspirated six to eight days before death are given in Table II. The diet contained 120 gm. of fat daily.

TABLE II

	RIGHT PLEURA	LEFT PLEURA	PERITONEUM
	mg. %	*mg. %*	*mg. %*
Phospholipid	50	73	105
Free cholesterol	26	41	48
Cholesterol as esters	39	54	64
Neutral fat fatty acids	102	271	604
Total lipid	217	439	821

COMMENT

Leucemias and neoplasms are the most common etiologic agents in nontraumatic chylous effusion. Of the malignancies, carcinoma of the stomach is the most frequent. Robinson[6] showed that ligation of the superior vena cava alone could produce chylous effusions, though ligation of both the right and left thoracic ducts would not. No thrombus or obstruction was found at this site in the present case. The anastomatic collateral lymphatic circulation and lymphaticovenous connections, which Lee[4] described as developing after ligation of the thoracic duct, prevent this accumulation of fluid. Though the larger collaterals were not demonstrated, the main branches of the thoracic duct (Fig. 2), and many of the smaller lymphatics (Fig. 3) in the abdominal organs were found permeated and partially obstructed by masses of degenerating tumor cells. This is said to result in an elevated pressure in the lacteals, with increased

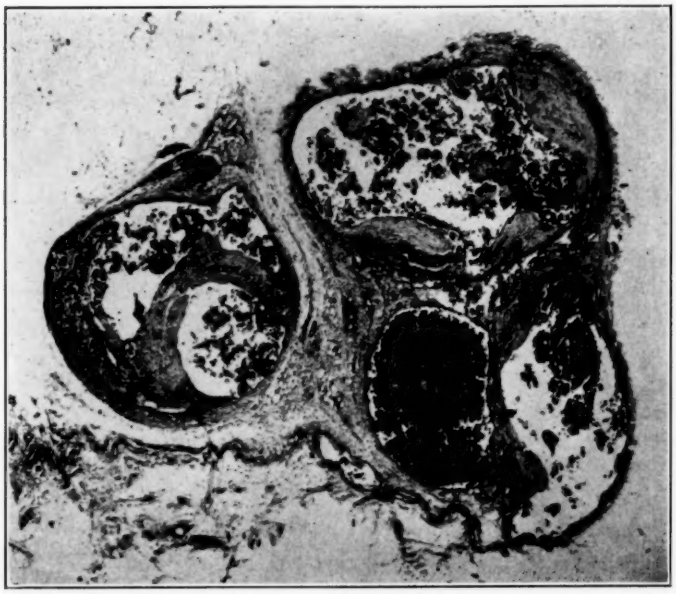

Fig. 2.—Thoracic duct near its termination showing multiple channels with masses of tumor cells and old thrombi in the lumina (×25).

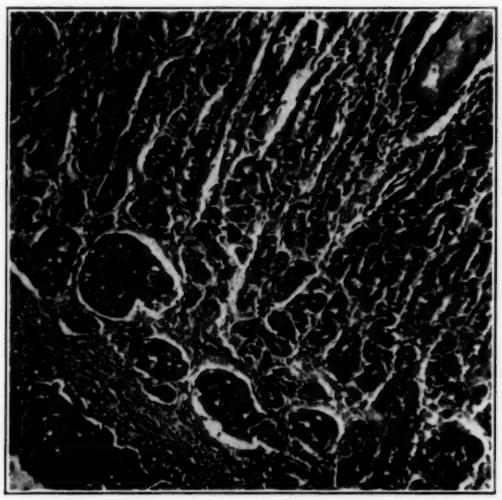

Fig. 3.—Stomach showing carcinoma cells blocking small lymphatics at the muscularis mucosae (×110).

transudation of the smaller fat particles through the intact walls, as well as rupture of the weaker ones and direct spilling of chyle into the free peritoneum. Such breaks are rarely proved. We can only speculate as to the cause of the variation in appearance and fat content in the various cavities. In traumatic chylothorax, the effusion occurs more often on the right, because of the anatomic arrangement of the duct.[1] In the present instance the most likely explanation is that the collaterals to the right thoracic duct, or the lymphaticovenous connections to the azygos vein, were the last to be occluded. That the

process was a progressive one is indicated by the finding at autopsy of frankly chylous fluid in both pleural cavities.

SUMMARY

A case of bilateral chylothorax with chylous ascites as the result of obstruction to lymphatics and the thoracic duct by carcinoma cells from a primary tumor of the stomach is reported.

Lipid analyses on fluid removed during life with the patient on a controlled fat intake show increasing fat content, chiefly in the neutral fat fatty acid fraction, in the right and left pleural and peritoneal cavities, respectively.

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HYPERINSULINISM DUE TO ADENOMA OF THE PANCREAS

Report of a Case

Robert G. Murphy, M.D., Cecil C. Dustin, M.D., and
Russel O. Bowman, Ph.D., Providence, R. I.

A CASE history of a man who suffered from hyperinsulinism due to adenoma of the pancreas is presented.

Seale Harris[1] in 1924 first described the condition of hyperinsulinism due to excessive secretion of insulin. This has been shown to have a great variety of manifestations, such as low blood sugar, hunger, sweating, nervousness, weakness, tremors, mental lapses, convulsions, delirium, and coma.

Numerous cases of tumors of the pancreas associated with the symptoms of hyperinsulinism have been reported. The first case proved pathologically was described by Wilder, Allen, Power, and Robertson[2] in 1927. It occurred in a 40-year-old physician who had attacks of unconsciousness with convulsions due to a carcinoma of the pancreas. An extract of one of the liver metastases acted like insulin when injected into rabbits; this proved the tumor cells capable of producing insulin.

Received for publication, December 31, 1938.

In 1926 Warren[3] reported 4 cases and collected 16 others from the literature.

Since Warren's paper other reports of hyperinsulinism have appeared.[4] These have been associated with carcinoma of islet cells, adenoma of the pancreas, or hyperplasia of the islet tissue. Reports have appeared in which there was hyperfunction of a morphologically normal pancreas, and a recent report claims relief of the hypoglycemia attacks after removal of a calcified tumor from the head of the pancreas.[5] Relief of hypoglycemic symptoms by appropriate surgical procedures, including removal of tumors and subtotal resection of the pancreas, has also been reported.

CASE REPORT

J. C., an Irish male, aged 60 years, was admitted to the Medical Service of Rhode Island Hospital after having been confused and disoriented for three hours. For a period of seven months previous to admission, he had had similar attacks about twice weekly. On these occasions, usually just before the evening meal, the patient became delirious, crossed and uncrossed his legs, and waved his arms about his head, occasionally falling. These attacks had been ascribed to alcoholism, though an alcoholic breath was never noted. There was no history of loss of consciousness, hunger pains, weakness, sweating, palpitation, precordial pain, or dyspnea. It had not been noted that food relieved attacks. He was said to have been unusually irritable, and experienced occasional tremors which did not precede attacks. At times his gait was ataxic.

Physical examination revealed a well-developed and well-nourished man lying in bed, confused and disoriented, but with no other complaint. He was unable to recognize common objects and was reluctant to answer questions. His temperature was 98° F., his pulse rate was 65, and his respiratory rate was 20. There was enlargement of the heart to the left, a systolic murmur over the precordium, loudest at the apex, and marked sclerosis of the radial arteries. His blood pressure was 172 systolic and 95 diastolic. The knee jerks, biceps, and triceps tendon reflexes were hyperactive on the right, and a doubtful Babinski was obtained on the right. Ophthalmoscopic examination of the optic fundi showed evidence of arteriosclerosis of the retinal arteries. There was no evidence of increased intracranial pressure.

Blood taken the morning after admission when the patient was fasting showed 43 mg. of glucose per 100 c.c., blood urea nitrogen of 11 mg. per 100 c.c., hemoglobin of 90 per cent (13.0 gm. per 100 c.c.), and white blood cell count of 7,550. Urine was slightly acid, had a specific gravity of 1.010, and was negative for sugar, albumin, and sediment. An electrocardiogram was not significant. Because of the low fasting blood sugar, the possibility of an islet cell tumor of the pancreas was considered. Further work was done to confirm the diagnosis and to rule out other possible causes of a hypoglycemia.

A phenolsulfonphthalein test showed 55 per cent excretion in two hours after intramuscular injection of the dye. Blood Wassermann and Hinton tests were negative. On subsequent days the following findings, exclusive of blood sugars, were obtained: carbon dioxide combining power, 54 volumes per 100 c.c. of plasma; serum calcium, 10.2 mg. per 100 c.c.; serum inorganic phosphorus, 3.4 mg. per 100 c.c.; total serum protein, 6.6 gm. per 100 c.c.; serum potassium, 19.5 mg. per 100 c.c.; whole blood sodium chloride, 388 mg. per 100 c.c.; serum cholesterol, 110 mg. per 100 c.c.; diastatic activity, 20 units (picric acid method); basal metabolic rate, minus 10.7 per cent. X-ray examination of the chest, examination of the gastrointestinal tract for duodenal deformity, and of the skull with special reference to the sella turcica, were all negative.

In Fig. 1 are given the fasting blood sugar values obtained from the patient during his stay in the hospital. The Benedict[6] method, very nearly specific for glucose and monosaccharides of like reducing properties, was used for blood sugar determinations. The patient had a blood sugar of 33 by the Benedict method, and of 43 by the Benedict-Lewis picric acid method.

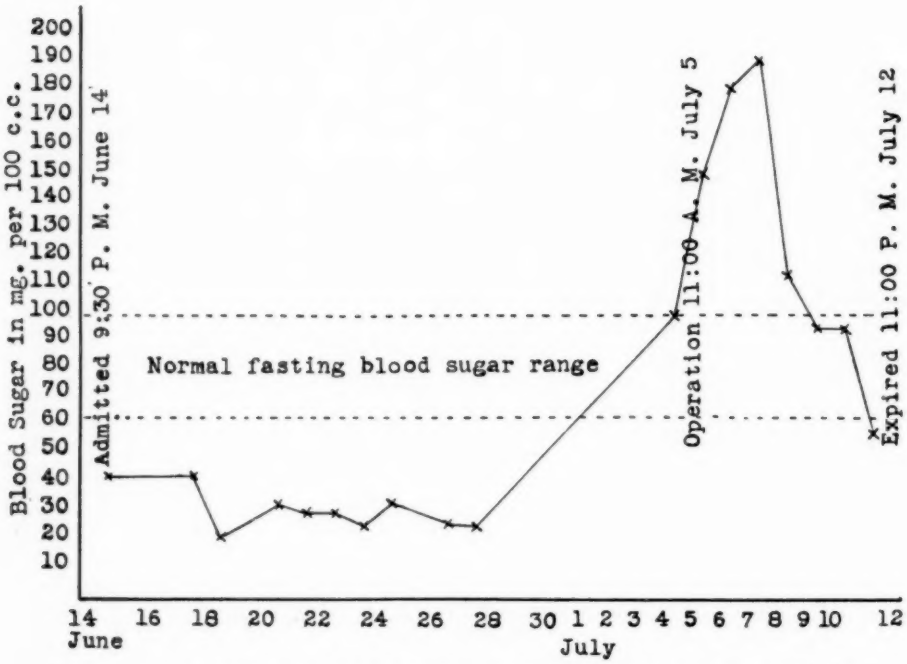

Fig. 1.—Fasting blood sugar values obtained on patient before and after operation.

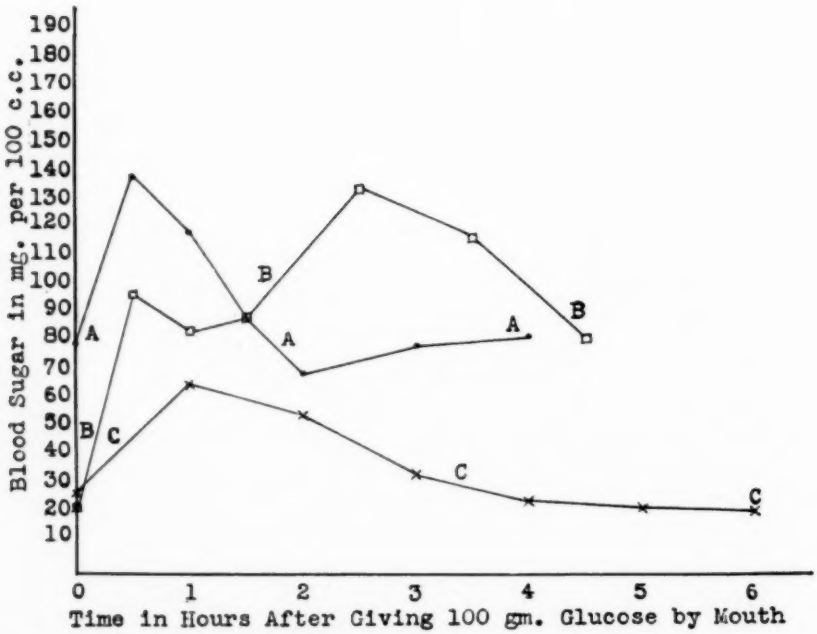

Fig. 2.—Glucose tolerance blood sugar curves.

●——● A Average normal curve.
□——□ B Patient on 6-19 after house diet.
×——× C Patient on 6-27 after high carbohydrate diet.

Two sugar tolerance tests, with an average normal curve, are given in Fig. 2. Curve B shows a maximum blood sugar response of 136 mg. per 100 c.c., reaching a maximum later than normal and returning toward the fasting value only after two and one-half hours. Previous to this the patient had been on house diet. There is evidence from curve B that storage of sugar as glycogen occurs slowly. For three days previous to the second tolerance test, curve C, the patient had been on a 200 gm. carbohydrate, 50 gm. fat, and 70 gm. protein diet. A marked change in the type of curve is noted. The maximum value is less than half the former, and the return toward the fasting level is more prompt. Although the final values of this curve are 22 and 21 mg. per 100 c.c., the patient had no symptoms or physical signs of an impending attack. The difference between curves C and B was probably due to training of the sugar-storing mechanism by a high carbohydrate diet previous to curve C.

Hyperinsulinism due to carcinoma, adenoma, or hyperplasia of the islet tissue of the pancreas was considered the probable diagnosis. Other causes for hypoglycemia, such as hypofunction of endocrine glands other than the pancreas (pituitary, adrenal, thyroid, gonads), liver disease, muscular dystrophies, severe infections, or prolonged starvation or fatigue, seemed to be excluded by laboratory tests, x-ray data, and physical examination.

An attempt was made to decrease the response of the pancreas by giving 10 units of regular insulin one-half hour after each meal, and a diet of 120 gm. carbohydrate, 80 gm. protein, and 188 gm. fat. At 9:30 P.M. the third day of this regime, the patient suddenly began to cross and uncross his legs, wave his arms, and cry "Stop, stop!" He was conscious but disoriented and confused. Each attempt to elicit a Babinski reflex stimulated the patient to greater activity and appeared to increase the severity of the attack. He was given 4 ounces of orange juice, and within five minutes became oriented and less confused; movement of his extremities ceased and could not be elicited by testing for the Babinski reflex. At 10:45 P.M. another attack of less severity occurred, but it was again controlled by giving orange juice.

The diet was changed to one of 200 gm. carbohydrate, 50 gm. fat, and 70 gm. protein.

A few days later a deliberate attempt was made to precipitate an attack by withholding the patient's breakfast. At 11:00 A.M. he showed marked tremors of the extremities, a fixed stare, profuse sweating, and pallor. He could not answer questions and became very restless, thrashed about in bed, and was restrained with difficulty. A blood sugar taken at this time showed 26 mg. per 100 c.c. Within three minutes after drinking 4 ounces of orange juice, the patient stopped thrashing about, answered questions, said he felt fine, and did not remember what had just happened.

He was transferred to the Surgical Service for laparotomy and exploration of the pancreas. He was given intravenous injection of 500 c.c. of physiologic saline and 100 c.c. of 50 per cent glucose twice the day before operation and once just prior to operation.

Under gas-oxygen-ether anesthesia a left upper rectus incision was made. The gastrocolic omentum was divided, the pancreas was exposed, and a small tumor was palpated in the head of the gland. This tumor was shelled out by blunt dissection.

Following the operation, the patient was given intravenous glucose and saline daily. His fasting blood sugars, as seen in Fig. 1, ranged from 100 the morning of operation to as high as 190 three days later, with gradual decline thereafter.

Urine sugars taken fasting were 1+ to 4+ for three days, and then became negative. Two days after operation his temperature rose to 101.8° F., his pulse rate to 126, and his respiration rate to 30. Examination of his chest showed signs of a bilateral bronchopneumonia which was considered to be a postoperative complication. His condition became progressively worse, and he died on the seventh postoperative day. During the postoperative course there were no symptoms or signs of hypoglycemia at any time. Permission for autopsy could not be obtained.

We are indebted to Dr. B. Earl Clarke, pathologist, for the photomicrograph (Fig. 3) and for the following report of the tumor removed at operation:

"*Gross Description:* The specimen consists of a rather spherical tumor mass which measures approximately 1 cm. in diameter. Part of its external surface is smooth and shiny as though it had been shelled from a capsule. However, elsewhere there are tags of tissue suggesting that it was adherent. The cut surface is rather grayish.

"*Microscopical Description:* There is an abundant dense hyaline stroma through which are scattered small areas of epithelial tissue, which vary in histology. There are several small ducts. There are also bits of glandular tissue like that of the external secretory part of the pancreas. There are, in addition, relatively large areas made up of narrow cords of cells in a close apposition to capillary blood vessels. These closely resemble normal islands.

"*Diagnosis:* Adenoma of the pancreas."

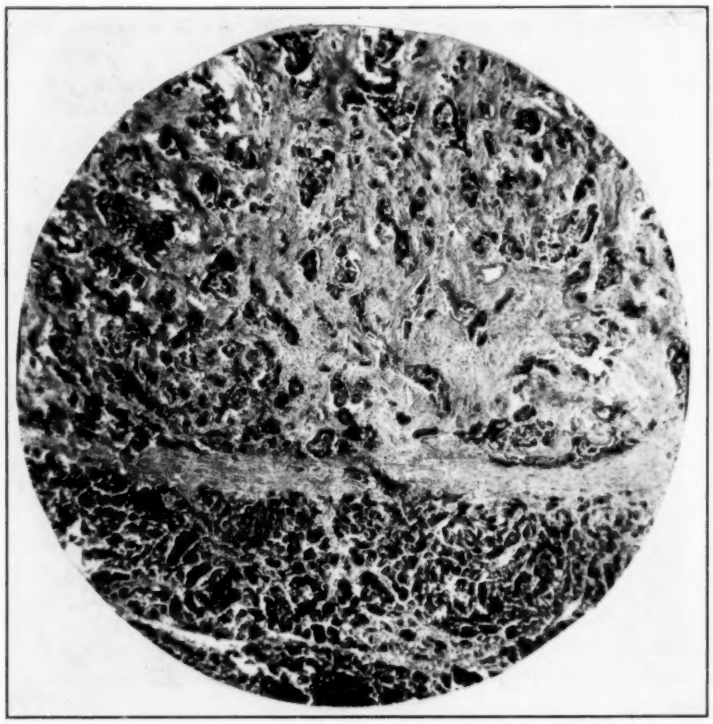

Fig. 3.—Low power photomicrograph showing the tumor (above) separated from the normal pancreatic tissue (below) by a distinct fibrous capsule. Note the abundant stroma and the variation of epithelial histology as described in the text. Hematoxylin and eosin preparation.

SUMMARY

A case of hyperinsulinism due to adenoma of the pancreas is presented with a description of hypoglycemic attacks and of the tumor removed by operation.

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THE PHOTOCOLORIMETRIC DETERMINATION OF VITAMIN A AND CAROTENE IN HUMAN PLASMA*

MARIAN STARK KIMBLE, PH.D., MADISON, WIS.

THE chief difficulty that has stood in the way of accurate quantitation of the Carr-Price reaction for determination of vitamin A is the very transitory character of the blue color that is produced. This comes to its height and begins to fade within a few seconds, and while the fading can apparently be somewhat retarded by a number of scrupulous precautions in the repurification and handling of the reagents, the necessity of their application so complicates the procedure as to restrict its practicability.

It is possible with the use of the Evelyn photoelectric colorimeter[1] to carry out the color-producing reaction with the cell in the reading position, and by watching the swing of the galvanometer, to follow the evanescent color to its definite point of maximum. In a recent communication[2] Dann and Evelyn have described the use of this instrument in a method of precision for the assay of vitamin A in oils. Dr. Charles May[3]† has developed a modification of the method for the determination of vitamin A in serum, using the *micro* unit of the colorimeter,[4] which is desirable if very small quantities of blood must be used. Because of the advantages afforded by the *macro* unit in convenience, speed, and accuracy of operation, it was felt that a more direct adaptation of the technique of Dann and Evelyn to blood analysis would be justified and would permit its wider application and study. Such an adaptation has been used in the orientation studies which follow.

Principle of the Method.—Blood plasma is extracted with alcohol and petroleum ether. The pigments dissolve in the alcohol, and carotene and vitamin A go quantitatively into the benzine layer. The yellow color of the carotene in the petroleum ether is read with filter 440 (blue) in the colorimeter as the basis for later correction of the blue color of the Carr-Price reaction, to which both carotene and vitamin A contribute. For the Carr-Price reaction the petroleum ether is aerated off and the residue is taken up in chloroform. Filter 620 (red) is used in the reading of the blue color. With the test tube cell containing the plasma extract in the reading position, the antimony trichloride reagent is added all at once, and the reading of the galvanometer recorded at the point of momentary stability which occurs at the height of color formation. After this, a reversal of the galvanometer swing indicates that fading has started. Since the rapidity of fading, rather than the point of color maximum, appears to be altered by ordinary impurities in the reagents, particular care in their repurification and anaerobic handling has not been found necessary with the use of the macroinstrument.

*From the Biochemical Laboratory, Department of Medicine, University of Wisconsin and State of Wisconsin General Hospital, Madison.

Received for publication, December 30, 1938.

†Dr. May has kindly furnished a manuscript copy of his procedure in the micromethod. His results have not been available up to this time of writing.

METHOD

Reagents.—

1. 95 per cent alcohol.

2. Light petroleum ether. Mallinckrodt's ether, B.P. not over 80° C. was redistilled, and the fraction boiling under 45° used in all of the earlier analyses here reported. Later this was replaced by Baker's C.P. special benzine, B.P. 20°-40° C.

3. Chloroform. Anesthetic or U.S.P. XI chloroform has been used without further drying or purification. Exposure to air has been kept at a minimum by opening quantities which last only for a short time.

4. Antimony trichloride reagent: 25 per cent in chloroform, weight in volume. Solution without heating has been obtained by pulverizing the chemical before weighing and then crushing it further with a footed glass rod in a wide-mouthed glass-stoppered brown bottle in which it is both weighed and stored. The reagent is kept at room temperature, to avoid precipitation and irregular changes in concentration. Both Merck's and Mallinckrodt's analytical grades have been used interchangeably. *Precaution:* Rinse all utensils which have contained antimony trichloride with fairly strong hydrochloric acid before washing, since water decomposes the salt with the formation of slimy, tenacious precipitates.

Colorimeter.—Evelyn photoelectric macrocolorimeter with filters 440 and 620.* A set of the specially selected ("S") absorption test tubes is reselected and marked to give uniform blank readings.

PROCEDURE

Three and one-half to 5.0 c.c. (preferably 5.0) of plasma from oxalated blood (one drop of 30 per cent potassium oxalate to 10-12 c.c. of blood) are used for a determination. The sample is measured into a narrow-necked, glass-stoppered 25 c.c. centrifuge tube† and an equal volume of 95 per cent alcohol and 12 c.c. of petroleum ether, accurately measured, are added. The stopper is sealed in place with a small drop of mineral oil. The contents are mixed by end-over-end inversion of the tube for ten minutes, and then centrifuged for a short time to aid in layering. The tubes have regularly withstood speeds of 1700-2200 r.p.m. Ten cubic centimeters of the supernatant petroleum ether extract are pipetted into a colorimeter tube and the yellow color read with filter 440 against a petroleum ether blank. The galvanometer reading for carotene is recorded as G_{440}, and the corresponding optical density (read from the L-G table accompanying the instrument) as L_{440}. The petroleum ether is then evaporated off in a current of warm dry air or carbon dioxide.‡ For petroleum ether boiling

*The colorimeter with necessary filters may be purchased from the Rubicon Company, 29 North Sixth Street, Philadelphia.

†Glass-stoppered centrifuge tubes of this kind have been obtained on special order through Eimer and Amend Company, specifying tubes similar to their catalogue No. 20061/6, but of 25 c.c. capacity.

‡Tanks of carbon dioxide with pressures too low to be used in the freezing of pathologic sections may be used for this purpose conveniently and without extra expense. Hence carbon dioxide has been used throughout most of the present work, though with determinations of blood A, aeration with room air proved to be equally satisfactory from the standpoint of recovery (see Table IV, Experiments 7 to 10). The carbon dioxide or air is delivered to a manifold through a bottle of calcium chloride.

under 45°, the 10 c.c. can be taken to dryness in about ten minutes with a stream of 6-7 L/minute, the tubes in a water bath at 40° to 43° C.

The outsides of the tubes are rinsed and dried, and the residue in each dissolved in 1 c.c. of chloroform. An antimony trichloride blank is set at 100 in the colorimeter with filter 620, and the center setting determined and allowed to become absolutely stable. The unknown tube with the chloroform extract is then put in place in the instrument, and 9 c.c. of antimony trichloride reagent is added rapidly down the side of the tube from a quick-delivery pipette. (For this purpose a 10 c.c. transfer pipette may be cut off from the end and recalibrated.) The galvanometer will oscillate briefly, as mixing occurs, and then move to the left to indicate a point of equilibrium which is transitory, but unmistakable. Then it will reverse its direction and slowly move off again to the right as the color begins to fade. The galvanometer reading at the maximum color is recorded as G_{620}, and its corresponding density as L_{620}. The maximum may persist for several seconds or for only one to two seconds, depending apparently mostly upon the freshness of the reagent. It is important to note, as pointed out by Dann and Evelyn,[2] that the *initial reading*, when actually obtained at maximum color, is the same with old or new reagents, although the *rate of fading* of the color may vary. This fact was borne out in the present study by comparisons of different reagents, to be discussed below.

CALCULATION OF RESULTS

The basis for the use of standard spectrophotometric formulas in the calculation of the results obtained with the photoelectric colorimeter—in effect an abridged spectrophotometer—is outlined by Dann and Evelyn in their discussion of the determination of vitamin A in oils.[2]

When the initial light intensity affecting the photocell is adjusted to give a full scale deflection of 100 points through a tube containing pure solvent only (blank), the galvanometer reading, G, obtained on insertion of the colored sample solution, will represent the percentage of light transmitted by the colored solution in terms of the uncolored blank. The density, L, is then equal to $-\log \frac{G}{100}$, or

$$L = 2 - \log G.$$

The L readings corresponding to given galvanometer deflections, as found in the L-G table accompanying the colorimeter, represent the density of the solutions essentially as they would be measured spectrophotometrically.

Vitamin A.—The *L value unit* here used for the expression of vitamin A in plasma is calculated directly comparable to the unit used by Dann and Evelyn for the vitamin A in cod-liver oil.[2] This value was in turn made as closely analogous as possible with the familiar spectrophotometric unit E (1 cm., 1 per cent, 620 mμ), and is written L (1 cm., 1 per cent, 620 mμ). Since the effective layer of solution through which the light passes in the Evelyn colorimeter is 1.9 cm. instead of 1 cm. (spectrophotometer), this difference is equalized by introducing the factor $\frac{1}{1.9} = 0.525$ in the calculation of the L value. In the formula of Dann and Evelyn,

$$\text{L value (oil)} = \frac{52.5}{M} \times L_{620}' \qquad (1)$$

where M = *mg.* of oil in the sample taken for analysis. Then in the final 10 c.c. of solution read in the colorimeter tube there are represented M/10 *mg.* of the original oil per cubic centimeter of solution, or $(1/1{,}000 \times M/10) \times 100 = M/100$ *gm.* per 100 c.c. of solution. Then the L value (oil) (1 cm., 1 per cent, 620 mμ) $= \frac{1}{1.9} \times \frac{1}{M/100} \times L_{620}$, which on solution yields formula (1) above.

In the case of blood, where the concentration of the vitamin is much lower than in oil, the sample for analysis is taken in terms of cubic centimeters, instead of mg. of material—i.e., 1,000 times as large in order to furnish readable colors. In this case, if A = c.c. of plasma used in an analysis, then A/10 = c.c. of plasma represented per cubic centimeter of final solution in the colorimeter tube, and 100 × A/10, or 10A = c.c. of plasma represented per 100 c.c. of solution. Then

$$\text{L value (plasma)} = \frac{1}{1.9} \times \frac{1}{10A} \times L_{620} = 0.0525 \times L_{620} \quad \text{---------- (2)}$$

Correction for Carotene.—Since the carotinoids in plasma also react with antimony trichloride to produce blue color in excess of that produced by the vitamin A, a correction for the carotinoids must be applied to the final calculation of the vitamin. This is done according to the relationship L_{620}/L_{440}, given by Dann and Evelyn for pure samples of carotene. Accordingly, the corrected formula for vitamin A in plasma becomes

$$\text{L value (plasma, corrected)} = \frac{0.0525\ [L_{620} - (0.14\ L_{440})]}{A} \quad \text{---------- (3)}$$

In the routine here adopted for blood analysis, A = 10 c.c. of an original 12 c.c. of petroleum ether extract from 5 c.c. of plasma—i.e., it represents $\frac{5}{6} \times 5$ c.c. of the original plasma. When these quantities are adhered to

$$\text{L value (plasma, corrected)} = \frac{0.0525\ [L_{620} - (0.14\ L_{440})]}{25/6}$$
$$= 12.6\ [L_{620} - (0.14\ L_{440})] \times 10^{-3} \quad \text{------- (4)}$$

If more or less than 5 c.c. of plasma are used in the original extraction, the appropriate change is made in the factor 12.6.

It is to be noted that the plasma values given in the accompanying tables represent, for convenience in expression, 1,000 times the actual L values calculated from formula (4), that is, they represent L value × 10.[3*]

Carotene Concentration.—If record of the concentration of carotene in the plasma as mg. per 100 c.c. is desired, this may be estimated from the G_{440} reading by reference to a standardization curve like that of Fig. 1. This is simply an experimental curve obtained by reading known dilutions of a sample of pure carotene in petroleum ether with filter 440 against a petroleum ether blank. The material used as standard in the present work was from a freshly opened ampoule of crystalline carotene prepared by the General Biochemicals, Inc., Division of the S.M.A. Corporation of Cleveland. Because of the difficulty of preparing strictly pure carotene, different samples may yield somewhat different standardization curves.

*Using the factors of Holmes and Corbet for pure vitamin A (Holmes, H. N., and Corbet, R. E.: J. Am. Chem. Soc. 59: ii, 2042, 1937) and that of Dann and Evelyn for equivalence of the L value unit, it is possible to transpose these figures for L value in blood to their approximate equivalents in terms of International Units. In this way it is estimated that a solution with an L value of 1 would contain something like 58,500 I. U. of vitamin A per 100 c.c.

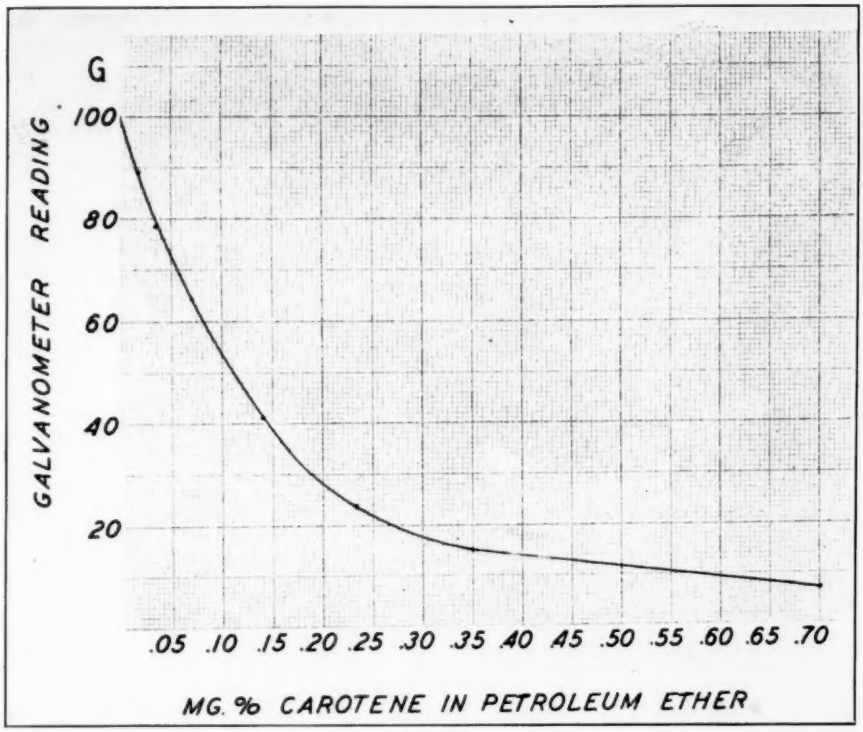

Fig. 1.—Standardization curve of pure carotene in petroleum ether. Percentage transmission at 440 mμ.

FACTERS FOR COVERTING READINGS FROM FIG. 1 TO CAROTENE CONCENTRATION IN PLASMA

WHEN A = 10/12 × X C.C. OF PLASMA EXTRACTED:	MULTIPLY CAROTENE READ FROM THE CURVE BY THE FOLLOWING FACTOR:
c.c. of plasma used:	f:
5.0	2.4
4.5	2.67
4.0	3.0
3.5	3.43

Dann and Evelyn found that the color of pure carotene in chloroform followed Beer's law, as does the blue color of the antimony trichloride reaction—i.e., that it showed a constant relationship between density (L) and amount of material in solution. They report an L value of 1,440 for their sample of pure carotene. Calculation of the L values ($\frac{52.5}{M} \times L_{440}$, where M = amount of the chromogen in each sample solution read) from Fig. 1 shows that the color of the carotene here dissolved in petroleum ether, rather than chloroform, has not followed Beer's law, i.e., the L value is not a constant. The values figure very close to 1,440, however, in the middle range of this curve, i.e., for galvanometer readings between about 24 to 64, which range covers most of the plasma values found.

RESULTS IN HUMAN PLASMA

Using the method here given, it has appeared that the substance in human plasma responsible for the Carr-Price reaction (corrected for the previtamin carotinoids), as shown in formula (3), is present in quite constant concentrations

in the plasma of healthy individuals, and that it evidently changes little under constant physiologic conditions, unless vitamin concentrates are being taken.

TABLE I

FASTING VERSUS POSTPRANDIAL BLOOD

SUBJECT	BLOOD TAKEN	CAROTENE MG. %	A L VALUE × 10^3
1	a. Fasting		
	b. Two hours after meal high in butter, cream, and milk. Heavy lipemia	a. 0.20 b. 0.23	a. 2.38 b. 2.32
2	a. Fasting		
	b. Two hours after meal including bacon, butter, milk, and cream. Moderate lipemia	a. 0.25 b. 0.26	a. 2.69 b. 2.51
3	a. Fasting		
	b. Six hours after breakfast and three hours after lunch including fruit, lettuce, butter, milk. Moderate lipemia	a. 0.19 b. 0.18	a. 1.66 b. 1.60
4	a. Fasting		
	b. Two hours after breakfast with egg, butter, and cream, plus 1 teaspoonful of *haliver oil.* Moderate lipemia	a. 0.14 b. 0.16	a. 3.50* b. 9.11
5	a. Fasting		
	b. Two and a half hours after breakfast with fruit, egg, butter, and cream, plus 1 teaspoonful of *haliver oil.* Moderate lipemia	a. 0.15 b. 0.14	a. 2.76* b. 6.00

*High fasting values in these cases had resulted from previous dosage with haliver oil, the last doses having been taken twenty-six and twenty-seven hours before the present experiments.

Fasting Versus Postprandial Values for Blood A.—It was observed (Table I) that, although a very large dose of the vitamin—teaspoonful of haliver oil—more than doubled the A in an already high blood within two hours, ordinary meals did not increase the concentrations of either carotene or A in the plasma within two to six hours, even though lipemia was definite in the postprandial samples. Hence the following studies are not restricted to fasting blood, but include as well samples taken either in midmorning or midafternoon.

Prompt Versus Delayed Analysis for Blood A.—Blood was taken with potassium oxalate as an anticoagulant, and the plasma was separated promptly by centrifugation. The determinations to be reported are about equally divided between those done immediately after the blood was withdrawn and those in which the separated plasma was kept in the refrigerator for from a few hours to overnight before analysis. The figures of Table II indicate that there was no significant loss of chromogenic power when the plasma was stored in the cold for a day before extraction.

TABLE II

DELAYED ANALYSIS

SUBJECT	CONDITIONS OF ANALYSIS	CAROTENE MG. %	A L VALUE × 10^3
1	a. Prompt analysis	a. 0.09	a. 1.75
	b. Plasma kept in refrigerator 20 hours	b. 0.09	b. 1.70
2	a. Prompt analysis	a. 0.25	a. 2.32
	b. Plasma kept in refrigerator 22 hours	b. 0.24	b. 2.35

Subjects Used.—Table III shows the figures for blood A and carotene obtained in a series of 30 male and 34 female control subjects in good health. The subjects were all connected with the clinical or laboratory staffs of the hospital and on uncontrolled dietaries, most of them felt from their descriptions to be at least adequate, and many more than the average liberal in A-containing foods. Correlation studies with dark adaptation measurements available on the same individuals will be included in later reports. The effect of large doses of A concentrates, with and without various supplements, is also being followed in a number of the subjects.

TABLE III

CAROTENE AND VITAMIN A IN PLASMA OF NORMAL CONTROLS

MALES				FEMALES			
SUBJECT	DATE	CAROTENE MG. %	A L VALUE × 10^3	SUBJECT	DATE	CAROTENE MG. %	A L VALUE × 10^3
1	3/22	0.12	2.60	1	9/12	0.17	1.42
	5/ 5	0.17	2.93	2	9/12	0.21	1.32
2	3/ 2	0.12	1.90		9/13	0.19	1.38
3	4/26	0.08	1.48	3	9/12	0.17	1.36
	10/11	0.18	1.79	4	9/13	0.19	1.59
4	4/26	0.05	1.59	5	9/14	0.34	1.58
5	4/29	0.10	1.75	6	9/14	0.17	1.27
6	5/ 4	0.10	2.02	7	9/17	0.14	1.13
7	5/ 4	0.15	2.18		9/21	0.10	1.13
8	5/ 4	0.07	1.82	8	9/17	0.18	1.19
9	6/17	0.15	2.28		9/21	0.14	1.37
	9/12	0.20	2.38	9	9/17	0.11	1.94
	9/12	0.23	2.32	10	9/19	0.29	2.22
10	9/14	0.29	2.40	11	9/20	0.14	1.69
	10/13	0.25	2.69	12	9/21	0.09	1.33
11	9/19	0.14	3.14	13	9/21	0.28	1.77
	9/21	0.17	3.30	14	9/26	0.24	1.33
12	9/19	0.18	1.53	15	9/26	0.09	1.11
13	9/19	0.20	1.76	16	9/26	0.15	1.17
14	9/19	0.14	2.04	17	9/26	0.14	1.33
15	9/20	0.13	2.07	18	9/27	0.21	1.19
16	9/20	0.13	2.05	19	9/27	0.18	2.00
17	9/20	0.14	1.66	20	9/27	0.24	1.32
18	9/20	0.15	2.07	21	9/30	0.32	1.92
19	9/27	0.20	1.89	22	10/ 7	0.24	1.69
20	9/27	0.30	1.76	23	10/ 7	0.17	1.62
21	10/7	0.20	2.36	24	10/ 7	0.16	2.04
22	10/7	0.30	1.55	25	10/11	0.20	1.65
23	10/12	0.14	1.76	26	10/11	0.24	1.80
24	10/12	0.12	2.52	27	10/12	0.22	1.51
25	10/14	0.25	2.32	28	10/12	0.28	1.40
26	10/14	0.21	2.62	29	10/13	0.19	1.66
27	10/17	0.18	2.96	30	10/13	0.14	2.82
28	10/17	0.18	2.20	31	10/13	0.23	1.97
29	10/18	0.10	2.66	32	10/17	0.20	1.25
30	10/25	0.14	2.44	33	10/17	0.25	1.84
				34	10/18	0.13	1.48
Average		0.166	2.18*	Average		0.187	1.56*
Standard deviation		0.059	0.50	Standard deviation		0.077	0.37
Coefficient of variability		0.36	0.229	Coefficient of variability		0.41	0.237

*See footnote on bottom page 1058.

Plasma Vitamin A in Healthy Controls.—The values in Table III, represented in Fig. 2, show an unanticipated but definite *sex difference* for concentration of the vitamin in plasma, the lower figures predominating in the female

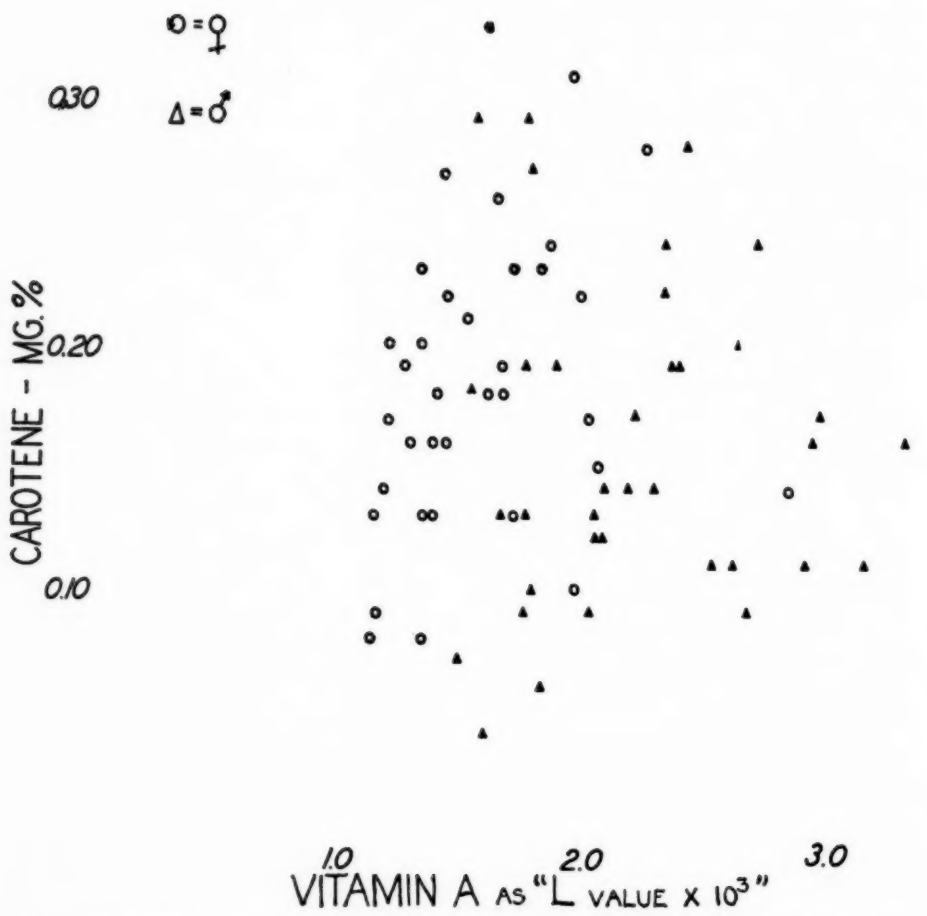

Fig. 2.—Lack of correlation between plasma vitamin A and carotene levels in healthy subjects. Δ = males; o = females. A sex difference in the concentration of A appears in the distribution of the points.

group. The average value found in 36 determinations on the 30 male subjects was *2.18* ($\times 10^{-3}$) L value units, with a range from 1.48 – 3.30, and a standard deviation of 0.50 (coefficient of variability* = 0.229). In 37 determinations on the 34 females, the corresponding average was *1.56*, the range from 1.11 – 2.82, and standard deviation of 0.37 (coefficient of variability = 0.237). Only 3 of the values for females fell above 2.0, while 22 were above this figure for the males. If this difference were due to relatively larger plasma: cell volumes in the female, carotene, as well, would be expected to vary in the same direction, whereas on the contrary, the carotene for these female subjects ran somewhat higher than for the males. If the physiologic demands for A in the female fluctuate with the hormone cycle—a problem which needs further investigation —one would expect to find greater variability in an unselected series of women than one of men, though this has not been definite in the present comparisons (coefficients of variability, above).

*Coefficient of Variability=the Standard Deviation expressed in terms of the mean value for the series, thus becoming comparable for series with their means at different levels.

Evidences of a sex difference in the ability to store and retain A in the liver of experimental animals were discovered in the literature after the foregoing observations were made on human plasma. In the protocols of Logaras and Drummond[5] it appears that female rats store more A in the liver under experimental conditions that tend to conserve the stores than do the males. Disappearance of A from the livers of depleted rats was slower in the females or in castrate males than in the male animals studied by Bult and Sorgdrager.[6]

Plasma Carotene in Healthy Controls.—There was a slight difference between men and women in the average figures for concentration or variability of plasma carotene in the above series of controls, with the tendency toward higher concentrations and greater variability in the females. The average for the males was 0.166 mg. per cent, and for the females, 0.187 mg. per cent. The values ranged for the males from 0.05 – 0.30, and for the females from 0.09 – 0.34, with coefficients of variability of 0.36 and 0.41, respectively. Fig. 2 shows that there is no consistent correlation between vitamin A and carotene values in the blood.

TESTS OF DEPENDABILITY OF THE METHOD

Reproducibility and Consistency of Figures.—Since pure crystalline vitamin A is not yet available for a known standard, judgments of the dependability of the present method must rest on tests of its consistency and reproducibility. Table IV shows the order of accuracy with which results have been reproduced in duplicate aliquots, and Table V the degree of constancy which has been noted at different times in the same individual under presumably constant physiologic conditions. The one significant change noted was a considerable increase in both carotene and vitamin A in subject 3, male (No. 6, Table V), following a late summer vacation. Experience with the biophotometer has suggested to some observers a seasonal variation with tendency to lower winter and higher summer readings. Examination of larger series will undoubtedly bring out further differences, and it is anticipated that more extended studies will reveal the basis for different groupings among the undifferentiated series of Table III.

From Table IV, experiments 5, 6, and 7, it is apparent that a significant part of the experimental error of the method is in the original extraction, rather than in the subsequent steps of the determination. The objectivity of the instrument and the ability it affords to make the readings at the absolute maximum of the color curve remove sources of inconsistency and uncertainty inevitable with the older methods, and clear the way for more comprehensive studies.

Stability.—The figures of Table II, and experiments 7 to 10 in Table IV, indicate that the vitamin is present in blood in a form that is relatively stable, since neither prompt analysis nor anaerobic handling appears necessary for consistent recovery.

Chromogenic Powers of Different $SbCl_3$ Reagents.—Because the accuracy of the Carr-Price reaction has often been challenged on the suspicion of variable chromogenic powers of different antimony trichloride reagents, or of the same lot of reagent under varying conditions, the experiments of Table VI were made to test this suspicion under the not at all rigid conditions of control of the present technique. Satisfactory checks were obtained in duplicate analyses made

TABLE IV

DUPLICATE CHECKS

EXPER. NO.	SOURCE OF MATERIAL	CAROTENE MG. %	A L VALUE × 10^3	REMARKS
1	Pooled plasma	0.17 0.17	1.59 1.60	Duplicates from pooled extracts
2	Pooled plasma	0.11 0.11 0.11	1.45 1.42 1.42	Triplicate aliquots from pooled extracts, using two different reagents
3	Pooled plasma	0.21 0.21 0.21	1.77- 1.77- 1.77+	Triplicate aliquots from pooled extracts, using same reagent
4	Pooled plasma	0.13 0.13	1.54 1.52	Duplicates from pooled extracts
5	Undiagnosed cachexia	---- ----	0.517 0.471	Duplicate analyses from separate extracts
6	Same patient as No. 5, five days later	---- ----	0.532 0.517	
7	Pooled plasma (a) = aerated with CO_2	(a) 0.15 0.15 (a) 0.12 0.12	(a) 1.51 1.50 (b) 1.56 1.56	Duplicate analyses from separate extracts
8	Control plasma (b) = aerated with room air	(a) 0.06 (b) 0.06	(a) 1.59 (b) 1.60	
9	Control plasma	(a) 0.10 (b) 0.10	(a) 1.48 (b) 1.48	Duplicates from pooled extracts
10	Control plasma	(a) 0.04 (b) 0.04	(a) 1.63 (b) 1.59	

TABLE V

SAME INDIVIDUAL AT DIFFERENT TIMES

NO.	CASE	SEX	DATES	CAROTENE MG. %	A, L VALUE × 10^3
1	Control	M	3/22/38 5/ 5/38	0.12 0.17	2.60 2.93
2	Control	M	6/17/38 9/12/38	0.15 0.20	2.28 2.38
3	Control	M	9/19/38 9/21/38	0.14 0.17	3.14 3.30
4	Ulcerative colitis	M	6/16/38 6/18/38	0.06 0.07	0.67 0.69
5	Control	M	9/14/38 10/13/38	0.29 0.25	2.40 2.69
6	Control	M	4/26/38 10/11/38	0.08 0.18	1.48 1.79
7	Control	F	9/12/38 9/13/38	0.21 0.19	1.32 1.38
8	Control	F	9/17/38 9/21/38	0.18 0.14	1.19 1.37
9	Control	F	9/17/38 9/21/38	0.14 0.10	1.13 1.13
10	Diabetic	F	5/13/38 5/16/38	0.096 0.096	2.29 2.38
11	Cachexia	F	4/ 1/38 4/ 6/38	---- ----	0.494 0.524

TABLE VI

CHECKS ON ANTIMONY TRICHLORIDE REAGENT

EXPER. NO.	SOURCE OF MATERIAL	$SbCl_3$ REAGENT USED	A L VALUE $\times 10^3$	SOURCE OF THE $SbCl_3$
1	Control blood	a. Solution 8 days old	2.28	Merck's reagent, mfg. date unknown
		b. Solution 4 weeks old (In refrig. between use)	2.34	Merck's reagent, mfg. date unknown
2	Diabetic blood	a. Solution one day old	1.17	Merck's reagent, mfg. date unknown
		b. Solution 2 months old (Kept at room temp.)	1.11	Merck's reagent, mfg. date unknown
3	Pooled extract	Solutions made from different known stocks of $SbCl_3$ (Kept at room temp.)	1.45 1.42 1.42	Stock No. 1: Merck's reagent, date: 32587 Stock No. 2: Merck's reagent, date: 33427
4	Pooled extract	Same conditions as in Experiment 3	1.54 1.51 1.54 1.52	Stock No. 1, as above Stock No. 2, as above Stock No. 3: Mallinckrodt's analyt. 3624, control G.N.H.1

with old and new solutions from the same reagent stock, and with antimony trichloride on hand from different stocks.

SUMMARY

The Dann and Evelyn photocolorimetric determination of vitamin A in oils by the Carr-Price reaction has been adapted for the assay of 3.5 to 5.0 c.c. of plasma. The technique differs from a prior microadaptation by C. D. May in using the Evelyn macro- instead of the microcolorimeter. With the use of this instrument a number of material simplifications in procedure have been found safe, with recoveries reproducibly consistent under a variety of conditions.

Results for plasma carotene and vitamin A are given for 30 men and 34 women in good health. While the figures for carotene were slightly higher on the average in the female group, those for vitamin A were found distinctly higher for the males than for the females, with averages 2.18 and 1.56×10^{-3} "L value" units roughly equivalent to 127 and 91 I.U. of vitamin A per 100 c.c. of plasma, respectively. Coefficients of variability for the vitamin values in the two groups were 0.229 and 0.237.

Vitamin A appears to be present in the plasma in a relatively stable form, and to vary little under constant physiologic conditions in individuals who are not taking oil concentrates. More extended studies will doubtless reveal different classifications among general groups of a healthy population.

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EFFECTS OF ANESTHETIC DRUGS UPON RATS TREATED WITH SULFANILAMIDE*

JOHN ADRIANI, M.D., NEW YORK, N. Y.

SULFANILAMIDE therapy has invaded practically every field of medicine. Surgical patients frequently receive the drug before operation. The anesthetist is often confronted with a patient who has recently had or is undergoing intense sulfanilamide therapy. Recent reports of toxic effects from the drug and particularly the untoward reactions when given with magnesium sulfate or other depressing drugs,[1] have focused attention upon the possibility of dangerous sequelae when narcotics or anesthetics are to be administered during the time when sulfanilamide is present in the body.

The pigmentation or skin color changes that follow administration of sulfanilamide, at first thought due to alteration in the oxygen-carrying pigment, and later observations revealing hemolytic anemia or granulocytopenia, have directed attention of anesthetists to possible asphyxial reactions.[2] Circulatory effects in man during anesthesia have suggested this in a few observations not yet confirmed. More recently, Marshall[3] has shown that toxic doses depress the cortex of the dog, producing results which suggest a decorticated animal. Many hypnotic and anesthetic drugs act upon the cortex. Therefore, attention is also directed toward unfavorable results that may be associated with cortical depression.

The present study was undertaken to observe the reactions of animals treated with sulfanilamide and subjected to anesthesia with the various drugs in current use. Rats were selected as the experimental animal. The drug is detoxified similarly in rats and human beings, by conjugation with acetic acid.[3] The toxicity in rats is low and the absorption slower than in human beings when the drug is given orally or subcutaneously.[4] The animals used in this study were a special strain of white rats, 6 months old, weighing approximately 200 gm. each.

The administration of sulfanilamide to a number of animals is simplified by preparing solutions of the drug. Such solutions were prepared with normal saline as a diluent. Injection was made intraperitoneally with solutions at constant temperature of 37° C. Doses employed amounted to 0.5-1.0 mg. per gram of rat daily.[5] The animals were treated for three consecutive days before anesthesia was administered. Determinations of sulfanilamide in the blood were not undertaken.

The following observations were completed using treated rats (1.0 mg. per gram) with a similar number of untreated controls.

*From the Division of Surgery, Department of Anesthesia and Laboratory of Experimental Surgery, New York University College of Medicine, New York.

Received for publication, January 11, 1939.

1. *Reaction to Asphyxia.*—Twenty rats were used with 20 untreated controls. On the third day of treatment, three hours after sulfanilamide had last been given, the rats were placed under a large bell jar and nitrogen added continuously but slowly. With the lowered oxygen tension dyspnea was first observed without marked cyanosis. The degree, time of onset, and resultant effects were similar in the treated and untreated groups. Symptoms of asphyxia were marked in all rats after eight minutes' administration of nitrogen. After five minutes' further observations, nitrogen was again added until all the animals showed syncope. The concentration of nitrogen was not determined. Oxygen was then added to replace the nitrogen. Recovery of all rats occurred in three minutes. Recovery was similar in the treated and untreated rats and there was no apparent difference between the groups during the subsequent three hours when they were closely observed. No difference was noted in their behavior during the next several days.

2. *Reaction to Excess Carbon Dioxide.*—Twenty treated and the same number of untreated rats of the same size and age were placed in a bell jar. Carbon dioxide was gradually added to slowly displace the air. All the rats showed a well-defined hyperpnea in less than one minute. The flow of carbon dioxide was continued ten minutes, or until respirations were depressed and the rats became unconscious. Rate and depth of respirations and the onset of unconsciousness were similar for treated and untreated rats. Anesthesia with carbon dioxide was maintained for one to two minutes. Respirations of the treated animals appeared to be predominantly abdominal, while in the untreated rats they were more of the intercostal type. After the bell jar was removed, recovery was no different in either group. No delayed reactions were noted.

3. *Reactions to Nitrous Oxide Narcosis.*—Ten treated and 10 untreated rats were placed in a bell jar, and nitrous oxide was gradually admitted. After eight and one-half minutes all animals were unconscious. Every animal exhibited marked cyanosis and a well-developed hyperpnea. Anesthesia was maintained for four minutes. Recovery was permitted by quickly introducing oxygen. The onset of unconsciousness, hyperpnea, cyanosis, and the signs and symptoms of narcosis were identical in all animals. Results were very similar to those observed in the experiment where nitrogen was used to produce asphyxia. None of the treated or untreated animals died.

4. *Reactions to Ether Anesthesia.*—Twenty treated rats were placed in a bell jar with 20 controls, and oxygen saturated with ether vapor was slowly added. After five minutes all rats were unconscious. They were maintained in this narcotic state for five minutes, during which time treated and untreated rats responded similarly. Ether was then added until (after four to six minutes) respiratory arrest occurred. Reactions of treated rats and the controls were similar, and there was no difference in the recovery. An identical number of the treated and the controls required artificial resuscitation. All rats recovered completely.

5. *Reactions to Cyclopropane Anesthesia.*—Eighteen treated rats were placed in a bell jar with 18 controls, and cyclopropane was slowly added. Within three and one-half minutes, all the rats were anesthetized. The onset

TABLE I

DRUG	DOSE MG./KG.	TREATED						UNTREATED					
		ANIMALS	HYPNOSIS ONSET	HYPNOSIS DURATION	ANESTHESIA ONSET	ANESTHESIA DURATION	MORTALITY	ANIMALS	HYPNOSIS ONSET	HYPNOSIS DURATION	ANESTHESIA ONSET	ANESTHESIA DURATION	MORTALITY
Group 1													
Thio-ethamyl	60	18	5.0	*	3.3	2 hr. +	30%	18	5.6	1-2 hr.	--	--	0
Group 2													
Thio-ethamyl	60	4	4.0	1-2	--	--	0	4	3.2	1-2	--	--	0
Group 3													
Evipal	80	10	2.7	26.7	5.5	14.3 min.	0	10	3.1	14 hr.	--	--	0 (1)
Pentothal	50	10	2.4	*	1.9	3 hr. +	50%	10	3.3	1½ hr.	2	*	10% (2)
Pentothal	40	8	2.0	*	2.0	3 hr. +	60%	8	3	1-2 hr.	--	--	0 (3)
Amytal	70	9	6	*	9	4 hr.	66%	9	6	2 hr.	9	3 hr.	33% (4)
Nembutal	40	16	2.1	*	4.0	5 hr. +	50%	16	4.4	3 hr. +	7.2	*	30%
Nembutal	20	10	5	1½-2	10	30 min.	0	10	6	1 hr.	--	--	0

*Undetermined—extended many hours.

Group 1.—Results following administration of thio-ethamyl 60 mg. per kg. body weight to a group of rats, one-half of which had been treated with 0.5 mg. sulfanilamide per gram body weight for three consecutive days.

Group 2.—Rats from the treated group in Table I that recovered from anesthesia and were allowed to rest for four days before reanesthetizing with thio-ethamyl.

Group 3.—Results from various barbiturates administered to rats treated with 0.5 mg. sulfanilamide per gram body weight and untreated controls.

(1) Controls not anesthetized.
(2) 20% controls not anesthetized.
(3) Controls not anesthetized.
(4) 66% controls not anesthetized.

of unconsciousness, the respirations and signs of anesthesia were similar in both groups. Anesthesia was maintained for five minutes. When no difference in behavior could be detected, cyclopropane was again added. Five minutes later one-half of the rats had respiratory arrest. The number was equally distributed among the treated and control animals. Air was added to effect recovery. Three of the controls did not recover. One treated rat died.

6. *Reactions to Chloroform Anesthesia.*—Fifteen rats were placed in the bell jar with 15 controls, and chloroform vapor was slowly added. Anesthesia, evident in three minutes, was maintained for eight minutes with no variation in effects and no difference in respiration for either group. Chloroform vapor was again added until half the total number of animals were at respiratory arrest. Two controls and one treated rat did not recover.

7. *Reactions With Tribromethanol Narcosis.*—A 3 per cent solution of tribromethanol in saline (at 37° C.) was prepared. One hundred fifty milligrams per kilogram were given intraperitoneally. The interval between injection and the loss of consciousness was noted as the interval of hypnosis. Anesthesia was determined by pinching the tail with a clamp and noting when pain reflexes were abolished.[6] Nineteen treated rats and 19 controls were tested. The interval of hypnosis averaged 1.9 minutes in the treated and 2.1 minutes in the controls. Onset of anesthesia was 2.1 minutes later in the treated and 2.0 minutes later in the controls. Anesthesia continued for an average of 17.6 minutes in the treated animals as compared to 15.2 minutes in the controls. Duration of the total period of unconsciousness differed but little for the two groups.

8. *Reactions to Injections of Derivatives of Barbituric Acid.*—Various barbiturates of the ultra short-acting and short-acting types that are frequently used clinically were administered intraperitoneally in a 2 per cent solution. The degree of hypnosis was determined by noting the reactions when the tail was pinched.[6] Methyl-butyl-ethyl-thiobarbituric acid (pentothal), isoamyl-ethyl-thiobarbiturate (thio-ethamyl), and N-methyl-cyclo-hexenyl-methyl-barbituric acid (evipal) were used in the form of their sodium salts. Isoamyl-ethyl-barbituric acid (amytal), the oxygen homologue of thio-ethamyl and pentobarbital, methyl-butyl-ethyl-barbituric acid (nembutal), the oxygen homologue of pentothal, were also employed. The amount of the drugs given, onset of hypnosis, and onset of anesthesia are tabulated in Table I. Duration of anesthesia which differed in the treated and untreated rats, very markedly in some instances, is also tabulated or explained in the text. These rats were given 0.5 mg. of sulfanilamide daily instead of 1.0 mg.

Eighteen treated and as many untreated rats were given 60 mg. per kg. (50 per cent M.L.D.),[7] sodium thio-ethamyl intraperitoneally. Hypnosis was evident in the treated rats in 4.2 minutes average, and in six minutes in the untreated group (Table I). Anesthesia, which continued more than three hours, was produced in all of the treated rats. None of the controls became anesthetized. Five of the treated group died without recovering from the anesthesia. Four surviving rats, shown in Table II, were allowed to recover

for four days. They were then given the same amount of sodium thio-ethamyl that had been administered to them previously. Controls were also injected. These four animals now reacted similarly to the controls, quite unlike their reaction when anesthetized during their treatment with sulfanilamide.

Pentothal, another thiobarbiturate, was given in doses of 50 and 40 mg. per kg., with a mortality of more than 60 per cent and 50 per cent, respectively, in treated rats, and 10 per cent and none in the untreated (Group 3).

Evipal, an ultra short-acting barbiturate, not a thio derivative, was administered, 80 mg. per kg. (40 per cent of M.L.D.).[8] All of the animals so treated recovered. The treated rats were anesthetized for an average of fourteen minutes, while for the controls only hypnosis was effected. Treated rats given amytal and nembutal likewise showed a marked difference to controls untreated with sulfanilamide (Group 3).

The animals that died following anesthesia with the barbiturates always developed a Cheyne-Stokes' type respiration and gray cyanosis. Autopsies revealed no significant gross findings. The blood was always very dark and did not coagulate.

Another group of 9 rats was given intraperitoneally sodium thio-ethamyl, 60 mg. per kg. of body weight, together with 0.5 mg. of sulfanilamide per gram of body weight. A similar number of controls received the same dose of the barbiturate only. Onset of hypnosis averaged four and one-half minutes in the control group, and none were anesthetized. In those treated with sulfanilamide, hypnosis developed at the same time, but in these animals the hypnosis deepened and lasted for an average of four and one-half hours. Controls recovered completely in this period.

Nine rats were given 60 mg. sodium thio-ethamyl per kilogram body weight four hours after receiving one injection of 0.5 mg. per gram of sulfanilamide. Anesthesia was not produced, but hypnosis was greatly prolonged over that of controls. Twenty-four hours after sulfanilamide treatment, the reaction to sodium thio-ethamyl anesthesia was similar to that of untreated rats. With 1.0 mg. per kg. per day the reaction was similar, the anesthesia prolonged and the mortality was as high as 60 per cent when 60 mg. thio-ethamyl was administered to rats so treated. The reaction was not obtained when less than 0.25 mg. per gram was used daily.

From these data it is obvious that rats treated with sulfanilamide react with little difference to volatile or gaseous anesthetics than do untreated controls. However, rats so treated react quite differently to injected anesthetic drugs than do untreated controls. The barbituric acid derivatives of the short and ultra short-acting groups, which are ordinarily used for surgical anesthesia, are not borne well by rats having had sulfanilamide. These barbiturates are thought to be detoxified in the liver. However, tribromethanol, which is conjugated with glycuronic acid, also in the liver, produces reactions very similar in treated and untreated groups. Delayed detoxification may not be the solution for the unusual narcotic effect of barbiturates. Barbiturates act upon the cortex and hypothalamus to depress the structures there.[9] Toxic doses of sulfanilamide in the dog likewise depress the cortex.[2] The combina-

tion of the two may greatly enhance the depression. Although the wide species variation to barbiturate reactions is established, it is suggested that the same combination may not be advisable in human therapy.

SUMMARY

White rats were treated with sulfanilamide 0.5 to 1.0 gm. per gram body weight daily for three days. These animals and a similar number of untreated controls were given various volatile, gaseous, and nonvolatile anesthetic drugs. They were also subjected to asphyxia and carbon dioxide excess.

Reactions to the volatile agents ether and chloroform, to the gases nitrous oxide and cyclopropane, and to carbon dioxide excess or oxygen want were the same in treated and untreated rats. There was practically no difference in the reaction to tribromethanol (avertin) in the two groups.

Rats treated with sulfanilamide and then given evipal, pentothal, thioethamyl, amytal, or nembutal, showed effects unlike untreated rats. The subanesthetic doses became anesthetic and often lethal. Anesthetic doses were usually lethal. The thio derivatives of barbituric acid were the worst offenders. Rats treated with sulfanilamide and allowed four days for recovery did not show this reaction. It was also less intense early in sulfanilamide therapy and more intense with increased dosage of the drug.

It is suggested that the combination of sulfanilamide and barbiturates may be unwise in human therapy.

Acknowledgment is made to Dr. E. A. Rovenstine and Dr. Charles L. Burstein, Professor and Instructor of Anesthesia, respectively, New York University College of Medicine, for assistance in this study and preparation of this report.

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477 First Avenue

LABORATORY METHODS

THE USE OF A REDUCING FACTOR OF PREGNANCY URINE IN THE DIAGNOSIS OF PREGNANCY*

DONALD E. BOWMAN, PH.D.
CLEVELAND, OHIO

IT IS generally agreed that biological assay affords an accurate means of diagnosing pregnancy. The most accurate and commonly employed biologic methods have the disadvantage of involving considerable time and expense. The length of time is especially undesirable in many cases. For these reasons, a purely chemical test as accurate as the biologic methods, but relatively simple in technique and economical in time and materials, should be of distinct value, since it would permit prompt diagnosis and at the same time obviate the need for the special facilities required by the biologic methods. In addition, a chemical test might be expected to yield results somewhat more quantitative in character than the biologic tests now employed and, therefore, aid in interpreting various abnormal conditions.

It was recognized at the inception of this study that preparations of urinary pregnancy hormones contained a reducing factor, and accordingly an attempt was made to utilize this in the development of a chemical test for pregnancy. At first a delicate qualitative reducing reaction was employed,[2] while later use was made of oxidation-reduction dyes as the indicator.[3, 4] The first test described by Visscher and Bowman[2] was found, under properly controlled conditions and in agreement with subsequent experiments of others, to yield an accuracy of 90 to 93 per cent, although with less care, and in the presence of certain interfering substances, the accuracy could be seriously reduced. In an effort to make the test more quantitative in character various oxidation-reduction dyes were next tried.[3, 4] Likewise attempts were made to separate the specific reducing factor from other reducing materials or interfering substances. To accomplish this, most of the reagents which have been reported in the literature as being capable of precipitating the urinary gonadotropic hormones were tried. In testing out the practical value of these several variations of the test, observations were made on urine specimens from well over a thousand cases. Although the test, improved by these modifications, fell short of the desired end in not being sufficiently quantitative or specific in character, it did serve to definitely correlate a reducing factor with the gonadotropic hormone.

*From the Department of Biochemistry, School of Medicine, Western Reserve University, Cleveland.

Aided by grant No. 379 from the Committee on Scientific Research of the American Medical Association.

Reported at the Meetings of the Sixteenth International Physiological Congress,[1] Zurich, Switzerland, August 17, 1938.

Received for publication, August 17, 1938.

It was found possible to put the observed reducing action on a definite quantitative basis by resorting to iodimetric titration, although it was obvious that the values obtained were the sum of the specific and other reducing substances in a given preparation. Therefore, electrometric studies were undertaken in order to observe the relative concentrations of the various reducing factors present.* It was hoped that by following the electrometric titration curves of various preparations in this manner, the most efficient means of purifying the desired reducing factor would be more readily apparent.

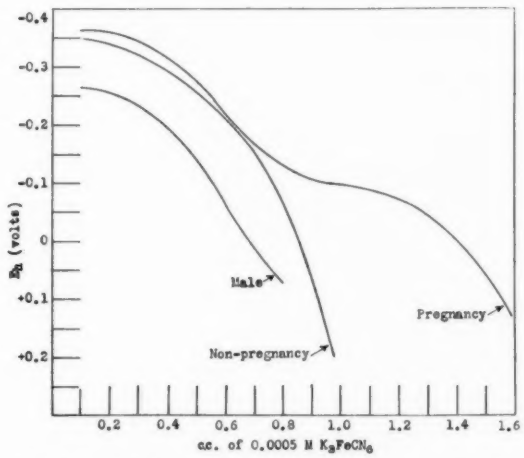

Fig. 1.—Typical oxidation-reduction titration curves.

Fig. 1 presents typical curves obtained during the course of the study which indicate the presence of the pregnancy reducing factor, as well as an interfering substance unrelated to pregnancy. Following various urinary preparations in this way, it was found that the interfering substances responsible for the upper portion of these curves could be eliminated by fractional precipitation with acetone held at a definite pH with a phosphate buffer solution. This method of separation was then utilized as the basis of a more simple titrimetric technique which has now been employed in a study of over 300 normal cases.

Method.—The details of the method now employed are as follows: A fresh specimen of urine, having a specific gravity of 1.020 or higher, is brought to a specific gravity of 1.020 with distilled water. Twelve cubic centimeters of the urine are then adjusted to a pH of 7.4 by adding 10 per cent sodium hydroxide, using bromthymol blue on a spot plate as the indicator. Nine cubic centimeters of acetone are added, the tube stoppered, and the precipitate centrifuged down. The supernatant fluid is then poured off and adjusted to a pH about 5.9, with 66 per cent sulfosalicylic acid, using the same indicator. If a precipitate forms at this point, it is centrifuged down and discarded. One cubic centimeter of 1 M. Sörensen's phosphate buffer, pH 5.90, is added to the fluid, followed by 18 c.c. of acetone. This is stirred several minutes, stoppered, and centrifuged for ten minutes. The supernatant fluid is drawn off by suction, carefully leaving the

*The author wishes to thank Dr. Henry Borsook, of the California Institute of Technology, for aid in the construction and use of the electrometric titration apparatus.

precipitate and the viscous, well-separated layer of fluid in the bottom of the centrifuge tube. The viscous fluid contains the precipitated phosphate salts in addition to the pregnancy reducing factor and a gonadotropic factor. The precipitate and the viscous fluid are dissolved in 5 c.c. of water. One cubic centimeter of this solution is pipetted into a test tube, avoiding an excess of any insoluble precipitate which may be present. (The use of 1 c.c. also permits a duplicate titration when this is desirable.) After adding a few drops of 5 per cent starch solution, this is titrated with 0.0005 N iodine in a water bath, maintained carefully at 38° C. The oxidation-reduction equilibrium is reached quite slowly, so that in this series the iodine was added in 1 c.c. quantities allowing five minutes between each further addition and continuing until a definite starch-iodine color remained at the end of the five-minute interval. Employing this technique, it has been found to be important that the original specimen had a specific gravity of 1.020 or higher, otherwise some false negative results might be obtained. Effort is being made to reduce this requirement.

TABLE I

RANGE OF TITRATION VALUES, TERMS 0.0005 N IODINE

	MAXIMUM TITER	MINIMUM TITER	AVERAGE TITER
	c.c.	*c.c.*	*c.c.*
Pregnancy	50	10	17.2
Nonpregnancy	5	1	2.33
Male	5	1	2.69

Table I gives the range of titration values obtained in this series when 0.0005 N iodine was used as the oxidant. It will be observed that there was a marked difference between the average titration values for the pregnancy and the nonpregnancy preparations. It may be further pointed out that, with the exception of 3 cases, the pregnancy and the nonpregnancy values had no tendency to overlap. In other words, the nonpregnancy titers did not exceed 5 c.c., while the pregnancy titers exceeded 10 c.c.

TABLE II

RESULTS OBTAINED WITH PRESENT METHOD

Correct positives	108
Correct negatives (female)	80
Correct negatives (male)	115
False positives	1
False negatives	0
Doubtful results	3
Total number of cases	303
Per cent of accuracy	98.4

Table II presents the data from this same series, arranged in a qualitative manner showing the distribution of cases.

DISCUSSION

The identity of this reducing substance is of considerable interest. This is especially true in view of its apparent association with the gonadotropic factor of pregnancy urine. Since the concentration of urinary gonadotropic hormones is the only well-established basis of a biologic test for pregnancy, a chemical test

for such diagnosis would be of more immediate value if it indicated the relative concentration of these hormones.

Therefore, it is of particular interest to note that a preparation of the gonadotropic factor of pregnancy urine obtained by repeated reprecipitation with the well-known acetone method has many properties in common with the viscous fluid obtained in the chemical pregnancy test here described. Both give positive Molisch reactions and have gonadotropic activity when injected in young female rats. In addition, the gonadotropic preparation exhibits oxidation-reduction properties similar to those shown by the reducing factor employed in the pregnancy test.

Since this paper was completed, Gurin, Bachman, and Wilson have made an important contribution to our knowledge of the chemical nature of the gonadotropic hormone of pregnancy urine. In a preliminary note[5] they presented evidence which indicated that the reducing substance contained a galactose component. In their first final publication[6] they describe a method of extracting and purifying the gonadotropic hormone and state that highly purified preparations contain a carbohydrate-polypeptide complex, which has the properties of a mucoid. It is of further interest to note that this product slowly reduces ammoniacal silver nitrate in the cold.

The association of the reducing factor and the gonadotropic hormones has been observed in various other ways. In addition to the series of cases reported one patient was followed for a period of three months just prior to pregnancy and again for a similar period beginning with the second month of pregnancy. The urine was examined three times each week, and the rate of excretion of the reducing substance was found to be quite constant in each period. The iodine titer during the period of nonpregnancy varied between 2 and 3 c.c., while during pregnancy it varied between 40 and 50 c.c.

Similar associations of increased quantities of the gonadotropic hormone and the reducing factor are observed in the urine of adult males with testicular tumor and also in the urine of young boys. Furthermore, the concentration of these two urinary factors appears to decrease at about the same time following delivery. Finally, it might be noted that placental tissue extracts containing the gonadotropic factor have been found to contain relatively large amounts of the reducing factor.

It would appear, therefore, that the employment of this pregnancy reducing factor should furnish a valid basis for a chemical test for pregnancy, provided interfering factors have been eliminated. It is particularly significant and important that positive results are obtained with this chemical test about two weeks before the biological tests become positive.

SUMMARY

A method of separating a reducing substance found in pregnancy urine from other urinary reducing materials is described. The titration of this reducing substance has been made the basis of a chemical test for pregnancy.

It is believed that the use of this reducing factor provides a valid basis for a chemical test for pregnancy since it occurs in increased quantities in pregnancy urine, and, furthermore, possesses many similarities with a gonadotropic factor.

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NEGATIVE SEROLOGIC REACTION FOR SYPHILIS IN NINE PATIENTS WITH INFECTIOUS MONONUCLEOSIS

JOHN H. MILLS, M.D., AND ELSA JAHN, BALTIMORE, MD.

BERNSTEIN[1] found the Eagle complement fixation test or the Eagle flocculation test for syphilis to be positive in 6 of 37 patients suffering from infectious mononucleosis. Hatz[2] reported a positive Wassermann reaction with negative Kline test in a patient with infectious mononucleosis.

Nine patients at the University Hospital were diagnosed as having infectious mononucleosis in which the clinical diagnosis was confirmed by characteristic hematologic findings and a positive heterophile antibody agglutination titer of $\frac{1}{256}$ or higher. In 5 of these patients the technique of Bernstein[3] was used. In the remaining 4 patients the rapid method of Straus[4] was used in addition to the Bernstein modification of the Paul-Bunnell test. The Kolmer complement fixation test and a flocculation test (Kline test in two instances, Kahn test in the remainder) were negative in every instance.

While chance may account for the discrepancy between these results and those of Bernstein and Hatz, these observations may be added to the growing literature on the subject.

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Received for publication, September 28, 1938.

STUDIES IN THE PREPARATION OF POLLEN EXTRACTS*

II. Analysis of the Sediment of Pollen Extract Obtained by Vacuum Filtration. Its Questionable Specific Excitant Activity

H. Harold Gelfand, M.D., George Flamm, M.D., and A. J. Heifitz, B. S.,
New York, N. Y.

IN A previous report by Gelfand, Flamm, Center, and Heifitz,[1] the authors were able to demonstrate that the occurrence of sedimentation and precipitation of pollen extracts under vacuum filtration is due to the withdrawal of carbon dioxide from the solution by the force of the vacuum. These phenomena were found to be in proportion to the force exerted and to the amount of carbon dioxide thus withdrawn from the buffered saline. The first appearance of a flaky sediment was observed at the measured vacuum pressure of 29.75 inches of mercury. It was further shown that the sediment redissolves upon the addition of carbon dioxide, and that the cloud and sediment reappear when the carbon dioxide is mechanically withdrawn. We suggested the use of carbon dioxide or air pressure filtration rather than suction filtration in order to avoid the possibility of this precipitation and clouding.

In this second report it is our purpose to describe the chemical composition of the sediment formed in pollen extract by the process of vacuum filtration and also to give an account of the activity of the sediment as proved clinically by direct skin tests on pollen-sensitive individuals.

ANALYSIS OF SEDIMENT

Fifty grams of dried and defatted high ragweed pollen were extracted with bicarbonate buffer solution saturated with carbon dioxide, 5 per cent extracts. The solution was divided into two equal parts. One-half (extract I) was sterilized by pressure filtration; the other (extract II), by vacuum filtration.

Analysis of Extracts

	EXTRACT I	EXTRACT II
Total nitrogen	0.690 mg. per c.c.	0.551 mg. per c.c.
Nonprotein nitrogen	0.340 mg. per c.c.	0.331 mg. per c.c.
Protein nitrogen	0.350 mg. per c.c.	0.220 mg. per c.c.
Protein nitrogen I	0.350 mg. per c.c.	
Protein nitrogen II	0.220 mg. per c.c.	
Difference in protein nitrogen	0.130 mg. per c.c.	

Upon analysis it was found that extract I showed a total nitrogen content of 0.690 mg. per c.c. and protein nitrogen of 0.350 mg. per c.c. Extract II showed a total nitrogen of 0.551 mg. per c.c. and protein nitrogen of 0.220 mg. per c.c.

*From the Department of Allergy, Gouverneur Hospital, New York.

It was, therefore, found that extract I contained an excess of total nitrogen over extract II, amounting to 0.139 mg. per c.c., and that the protein nitrogen* of extract I was greater by 0.130 mg. per c.c.

The sediment in extract II, resulting from vacuum filtration, was analyzed by the following method: The sediment was collected by decanting the supernatant extract and centrifuging the last portion. The extract was then washed free of inorganic constituents several times with water from which the carbon dioxide had been expelled by boiling, and then dried over phosphorus pentoxide for twenty-four hours in a vacuum desiccator. After this, the sample was weighed and its weight was found to be 0.2 gm. It was then analyzed by microprocedure for nitrogen, carbon, and hydrogen, with the following results:

Nitrogen	
Weight of sample	6.360 mg.
Net volume of nitrogen	0.145 c.c.
Pressure temperature, 765 mm. Hg	270° C.
Percentage of nitrogen	2.61
Carbon and Hydrogen	
Weight of sample	8.038 mg.
Weight of water	1.095 mg.
Weight of carbon dioxide	2.135 mg.
Weight of residue	5.528 mg.
Percentage of hydrogen	1.52
Percentage of carbon	7.24
Percentage of residue	68.8

It is evident from these experiments that the sediment obtained by vacuum filtration contained nitrogen, carbon, and hydrogen. The extract which had no sediment had a slightly higher protein nitrogen content.

DIRECT SKIN TESTS

After a sediment from ragweed extract in the sample under study was obtained by vacuum filtration, we proceeded to test it for ability to produce cutaneous reactions. Ten patients known to be clinically hypersensitive to ragweed, and who gave positive cutaneous reactions when tested with ragweed saline extract, were selected at random. Scratch tests were made on the inner surface of the arm in the usual manner, the skin being abraded and N/10 sodium hydroxide applied, followed by the application of a small amount of the sediment. A control scratch with N/10 sodium hydroxide alone was also carried out on each patient. All the subjects gave immediate strong reactions, of the positive wheal type, to the sediment (Fig. 1). The reaction of each subject was in direct proportion to the degree of sensitivity of the patient as previously elicited by the direct intracutaneous test with the usual saline extract of ragweed pollen. Thus Class "A" patients, when so treated, showed the largest wheals, and Class "C" the smallest.†

A similar number of patients known not to be ragweed sensitive were tested with the sediment, and their reactions proved to be constantly negative.

*Protein nitrogen equals phosphotungstate precipitate.

†Class A = most sensitive and Class C = least sensitive as determined by serial dilution tests.

COMMENT

The powdery sediment formed in ragweed pollen extract, which had been filtered by suction through a Seitz or Berkefeld filter and allowed to stand, has been analyzed quantitatively. The analysis revealed that the sediment contained nitrogen.

This sediment, after being dialyzed and dried, was used in testing patients known to be sensitive to ragweed, as determined by the use of saline extracts. They showed constant and positive reactions by the immediate formation of wheals.

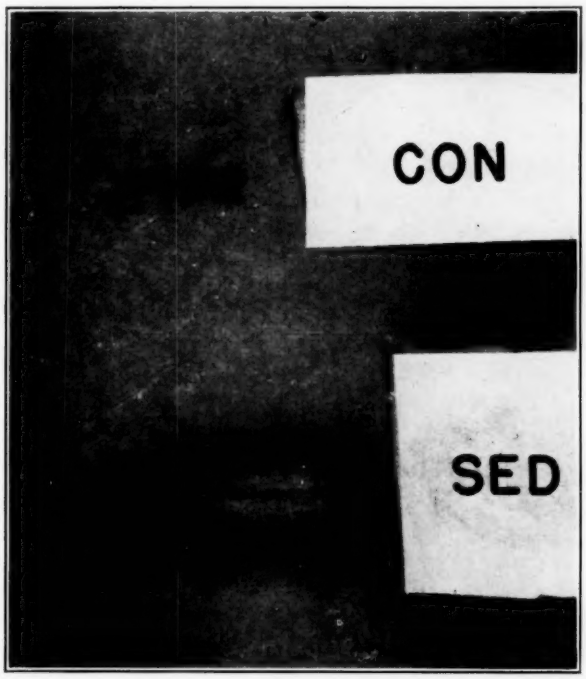

Fig. 1.

It is to be seen that in the process of vacuum filtration of ragweed extract, when precipitation and sedimentation occur, it is as a result of alteration in the colloidal solution because of the withdrawal of carbon dioxide by means of suction pressure. This resulted only when the extracting fluid used was the buffered saline recommended by Coca, in which excess carbon dioxide is allowed to bubble through before use.

We have ascertained definitely that alteration in the colloidal solution due to the withdrawal of carbon dioxide will produce the precipitation and sedimentation. Why some batches of pollen will give the sediment and other batches of pollen, although prepared in the same manner, will fail to give it, is unknown to us. One observes that the differences in protein nitrogen in analysis between part I (that sterilized by pressure filtration) and part II (that sterilized by vacuum filtration), give us a figure which for all intensive purposes is rather small and inconsequential in regard to protein nitrogen loss. Hence it is doubtful

whether that little loss of total or protein nitrogen will make any material difference in regard to the activity of the entire solution extract. It is also believed that the positive reaction observed from the dermal test performed by using the sediment as the allergen is probably due to absorption of protein by the sediment rather than the sediment proper.

CONCLUSION

In the process of preparation of pollen extracts using buffered saline solution (Coca extracting fluid) the vacuum pressure in the process of suction filtration causes a withdrawal of carbon dioxide with resultant precipitation and formation of sediment. We, therefore, recommend the use of pressure filtration by means of carbon dioxide or air filtration (10 to 20 pounds).

It has been shown that extracts prepared by vacuum filtration have a lower protein nitrogen content than those obtained by pressure filtration, but the amount of loss is so small as to be inconsequential and probably does not alter the activity of the entire solution extract.

The positive reaction obtained when a small amount of the sediment was used as a dermal test on known ragweed sensitive patients is undoubtedly due to the absorbed protein from the solution due to filtration.

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TESTS TO SHOW THE POSSIBLE INFLUENCE OF GREASY, ACID, AND ALKALINE TUBES ON THE WIDAL TEST*

With Subsequent Reference to Tubes Showing Acid Reaction

F. Smith, Kelowna, Canada

TESTS were made to see whether carelessness in preparing glassware might significantly affect the agglutinin-agglutinogen reaction.

Many checks were performed which coincided with the results herein quoted and paratyphoid A and B emulsions were checked with positive A and B serum. As the results are to show qualitative possibilities only, we quote just the results with *Br. abortus* and typhoid emulsions and their appropriate specific positive sera. This demonstrates the essential points without sacrificing clearness.

The author is aware that the results are explicable by exact chemical and physical rules, but it is chiefly with the technical aspect that this account is concerned.

1. Tests with serum known to be positive for *Abortus* with standard emulsion of *Br. abortus:*

*From the Okanagan Central Laboratory, Kelowna.
Received for publication, September 14, 1938.

SERUM DILUTION	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	CONTROL	DESCRIPTION
Br. abortus	+	+	+	+	+	–	–	–	Clean tubes, greaseless and neutral in reaction
Br. abortus	+	+	+	+	–	–	–	–	Tubes rinsed in soap solution and dried in air at room temperature
Br. abortus	+	+	+	+	–	–	–	–	Tubes coated inside with a transparent layer of paraffin
Br. abortus	+	+	+	+	–	–	–	–	Tubes boiled in soapy water, cleaned with a brush and rinsed 6 times in running water
Br. abortus	+	+	+	+	+	+	+	–	Tubes rinsed in normal HCl, dried in room; moisture wiped from rim

Having regard for the work of Michaelis, Beniash in 1911, and others, we controlled the acid agglutination with two extra tubes of saline and emulsion in acidified tubes as for the test. The controls were negative.

2. Tests with known positive typhoid serum and *B. typhosus:*

SERUM DILUTION	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	CONTROL	DESCRIPTION
B. typhosus	+	+	+	+	+	+	+	–	Tubes properly clean and neutral
B. typhosus	+	+	+	+	+	+	–	–	Tubes rinsed in soap solution, dried in air at room temperature
B. typhosus	+	+	+	+	+	+	+	–	Tubes rinsed in normal HCl, incompletely dried
B. typhosus	–	–	–	–	–	–	–	–	Tubes rinsed with normal NaOH, incompletely dried

The same serum and emulsions were used throughout in the case of each of these series.

It seems from these and the other tests we have done in this connection, that agglutination tests may be affected by greasiness, alkalinity, and acidity of tubes; that excess acidity can greatly enhance, and alkalinity can even make negative, a reaction.

With regard to the acidity of tubes and the possibility of using such for increasing a specific reaction, we are doing more detailed tests.

A SIMPLE INEXPENSIVE PIPETTING MACHINE*

JOHN H. MILLS, M.D., BALTIMORE, MD.

THOUGH there are inexpensive hand-operated pipetting machines on the market, it was felt that it might be worth while to describe a simple pipetting machine which can be made from a 2 c.c. syringe and a few odds and ends to be found about any laboratory. Such a machine has been in continuous use for the past ten months in the performance of over 5,000 complement fixation tests without the necessity of replacing any of the original parts. Reference to the diagram should make the details of its construction clear.

A 2 c.c. all-glass syringe (*2*) is mounted in a small wooden block (*1*) on a suitable baseboard about 8 inches by 12 inches in size. This block is channeled to take the syringe barrel snugly. A groove should be cut in the block near one end of the channel to take the flange of the syringe barrel. Narrow strips of metal (*3*) go over the syringe barrel and are fastened to the wooden block with screws so that the syringe is held perfectly rigid in the block. There must be no free movement of the syringe barrel whatever. Any similar mounting accomplishing this solidity is satisfactory.

The head of the syringe plunger is fitted into a slot in a metal sling or saddle (*4*) which is in turn bolted or soldered to a metal lever arm (*5*). The distance from the point at which the syringe plunger is mounted must be about 8 inches from the fulcrum point (*6*) so that pressure is not exerted in a sideward direction upon the syringe plunger as the lever is pressed. A slight bit of free play in the mount which holds the syringe plunger head also aids in preventing the plunger from sticking as the lever is pressed.

The lever arm (*5*) is pivoted at *6* by means of a small stove bolt with washers and lock nuts. Motion must be free. About this point the flat strip making the lever is twisted through a 90° arc at the middle (*7*).

At *9* is a rigidly mounted set screw which projects through a hole in the lever arm. This hole through which the set screw projects must be sufficiently large so that the set screw does not at any time touch the lever arm. It must, of course, be smaller than the diameter of the head of the syringe plunger. The set screw should be guarded with a lock nut (*18*). When the lever pulls the syringe plunger outward on its back stroke, the set screw limits the distance of its excursion. Adjusting this screw adjusts the volume delivered at each stroke of the lever. To insure proper filling of the syringe, a suitable number of rubber bands are looped around the set screw block and lever arm so that the syringe plunger is pulled back snugly against the set screw each time the pressure on the lever is released. Experimentation will determine the number of rubber bands necessary for this, as too much tension tends to

*From the University Hospital, Baltimore.
Received for publication, September 29, 1938.

make the syringe leak air and too little tension prevents accurate complete filling of the syringe to the proper volume.

To reduce friction, the end of the lever arm rides rather loosely upon some smooth thin object, such as a thin glass rod or piece of piano wire (*15*), which is mounted on the baseboard.

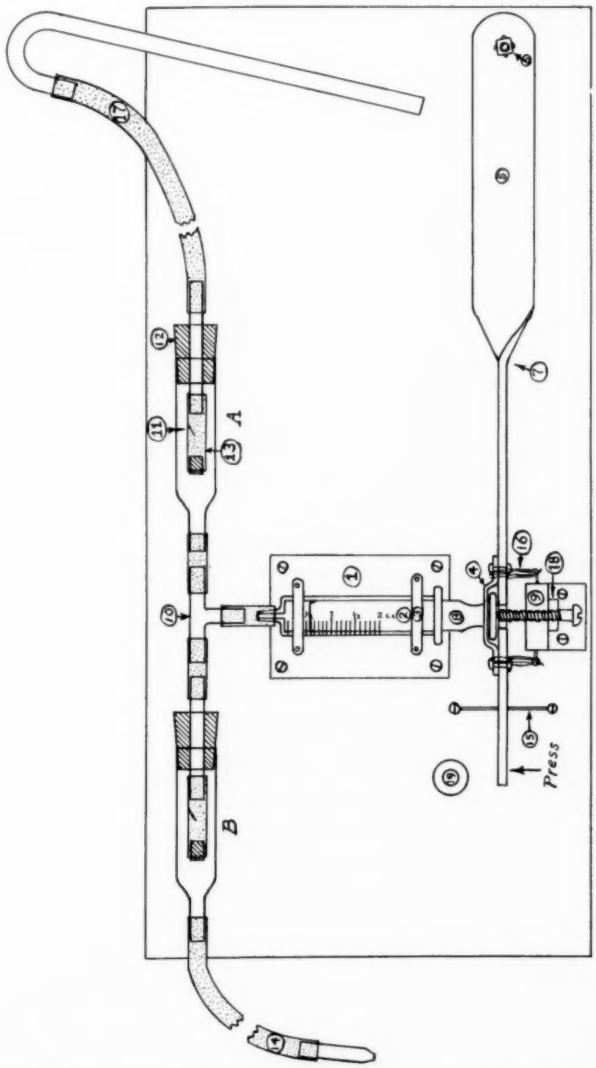

Fig. 1.

The tip of the syringe barrel is connected to a glass "T" tube (*10*). All arms of this "T" tube and all rubber connections should be as short as possible in order to reduce the total volume of fluid contained in the apparatus to a minimum. One arm of the "T" tube connects with the small end of the glass chamber *(A)*. The two valve chambers *(A)* and *(B)* are constructed from discarded 10 c.c. transfer pipettes. The end of the bulb is cut off as near the point where it begins to narrow so as to conserve as much of the straight portion of the bulb as possible. A small rubber stopper (*12*) is fitted into the

large end of this valve chamber. The stopper is pierced by a small bore glass tubing which projects ½ inch from either end of the stopper. On the end of the glass tube, which projects within the chamber, an ordinary Bunsen valve is fitted (*11*). A Bunsen valve is merely a piece of gum rubber tubing with a slit approximately ¼ inch long, cut either parallel to the long axis of the tube or at an angle to the long axis (*11*). A straight smooth slit may be cut with a wet razor blade while the tube is pinched flat, cutting at the sharp bend in the tube. This is the valve commonly used on laboratory wash bottles. Other connections are shown in Fig. 1.

In constructing the valve chambers a few millimeters should be left between the ends and side walls of the Bunsen valves and the chamber walls to allow for slight motion of the valves in opening.

In operation pressure on the lever is exerted with either hand, and the delivery tube is held in the other hand. To insure that all air is removed from the system, the delivery nozzle may be connected with a water vacuum pump to produce a slow flow of fluid through the apparatus. The lever arm is pumped a few times while the fluid flows. This removes the air from the syringe chamber.

Mounting the whole apparatus in a vertical position with the tip of the syringe upward prevents collection of air in the syringe while the apparatus is in use. In such a case, extending the lever arm 8 inches beyond the fulcrum point and using this end as the point at which pressure is exerted during operation will permit of downward instead of the more inconvenient upward pressure. When space is conserved by using glass tubing of internal diameter of not over 4 mm. and rubber tubing of correspondingly small diameter, and all connections as short as possible, the total volume of the apparatus can be kept to less than 30 c.c., thus conserving reagents to be pipetted.

The volume delivered is checked before and after each run by delivering the requisite number of strokes into a small volumetric flask. Readjustment has been found rarely necessary, but is easily made. In calibration, the same speed of stroke, the same amount of tension on the lever, and the same length of tubing in the system should be maintained as is to be used in practice. Washing is easily accomplished by connecting the delivery nozzle with a water pump and filling the reservoir with washing liquid.

The reservoir from which fluid is pipetted should not be placed at too high a level to prevent the fluid pressure created by the siphon to force the valves open. Simple inspection of the apparatus when filled with fluid will readily determine this. The delivery tube (*14*) should be of thick-walled catheter tubing, about 20 F gauge, to prevent variations in volume delivered due to collapse of the side walls of this tube. A finger rest (*19*) may be screwed to the baseboard.

A hand-operated instrument offers the advantage of extreme flexibility in rate variation which is not pressed by electrically driven devices and can be operated as fast as the electrical pipetting machines. The advantage of economy is obvious.

A NEW METHOD FOR CLEANING AND STERILIZING KAHN OR WASSERMANN TUBES

N. O. Gunderson, M.D., and C. W. Anderson, B.S., Rockford, Ill.

THE cleaning and sterilizing of Kahn tubes, as it is carried out in various laboratories, is a slow and laborious process. Many laboratories do not have the time or the personnel required to clean every tube with chromic acid cleaning solution before it is used in making the test. Obviously, the difficulty in the usual methods lies in the fact that only 10 to 12 tubes can be handled in one operation. This also introduces the personal equation to such a degree that results in some cases may be quite unsatisfactory. As an alternative, this laboratory has been using stainless steel wire-cloth baskets, 6 inches by 6 inches by 3 inches in size, capacity 140 to 150 tubes, for handling the tubes in cleaning, rinsing, and drying operations. The baskets can also be used for cleaning 4 inch, 5 inch, and 6 inch test tubes as well as glass slides.

After the Kahn tests have been completed, the tubes are transferred directly from the Kahn racks to the stainless steel baskets. All tubes should be packed uniformly, either with the open end upward or downward, to facilitate rapid rinsing. They are then rinsed several times in a warm alkaline detergent, such as trisodium phosphate, after which they are rinsed in tap water and immersed in chromic acid cleaning solution a minimum of four or five hours, and preferably overnight. They are then given at least two rinses in each of three separate portions of fresh tap water, using not less than 5 quarts each time. The pH of the third portion of tap water should be checked occasionally. After this, they are rinsed twice in distilled water and the basket of tubes is placed in a drying oven at 120° C. All tubes which have contained whole blood should be washed and brushed separately in an alkaline detergent before being placed in the baskets for immersion in cleaning solution. Very satisfactory results can also be obtained by first removing the blood clots, then packing tubes in the baskets and immersing in a strong solution of trisodium phosphate, allowing them to remain overnight. The following morning rinse several times in tap water, and after giving them a careful inspection, they may be placed in the acid cleaning solution.

Laboratories using over 500 tubes per day might find it advantageous to use larger baskets, 6 inches by 6 inches by 6 inches. In this case one layer of tubes should be packed open end downward and the top layer with open end upward to facilitate rinsing. Baskets, 6 inches by 6 inches by 3 inches, are easily made up from square foot sections of the stainless steel-wire cloth. Fig. 1 illustrates a simple method of forming the basket.

Received for publication, October 3, 1938.

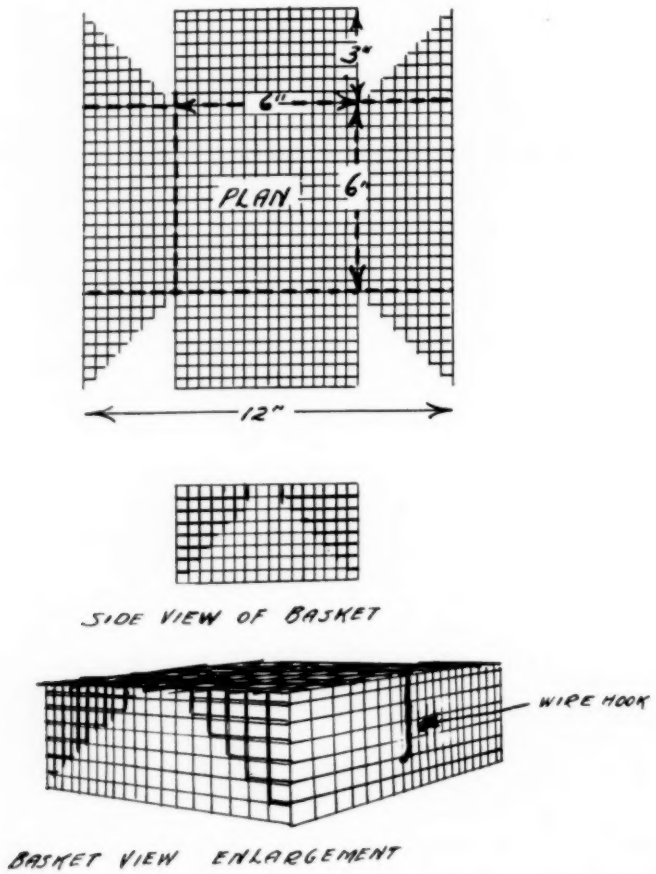

Fig. 1.

Three-inch triangular sections are cut from each corner of the square of cloth, as indicated by the diagram. After removing these pieces, the outer edges of the wire may be turned under by putting the cloth through a tinner's crimping machine, thereby removing sharp edges. The basket is then formed by bending the cloth, as indicated by the dotted lines, bringing the remaining triangular section up on the outside of the square end section. This gives added strength to the basket. The loose ends are hooked over into the wire permanently forming the basket. A top is made from a 6 inch square section of the same material and held in place with short sections of stainless steel wire formed into hooks. A more satisfactory basket could be made up by electric welding the end sections rather than hooking them together. Satisfactory tongs can be made up of heavy stainless steel wire for handling the baskets when they are immersed in the acid and during subsequent removal. This laboratory has been using a double pronged ⅛ inch lead coated wire hook, 12 inches long, with the upper end twisted to form a handle.

The wire cloth* used is a 4 mesh stainless steel No. 304, 18 per cent chromium, 8 per cent nickel. Corrosion tests of the wire cloth indicated a loss of ap-

*Manufactured by W. S. Tyler Co., Cleveland, Ohio.

proximately 0.1 of 1 per cent in one hundred hours at 20° C. when the cleaning solution was made according to the formula given in *The Kahn Test*, by Dr. R. L. Kahn, i.e., 252 c.c. concentrated sulfuric acid, 300 c.c. water, and 60 gm. potassium dichromate. The corrosion rate is increased at higher temperatures and decreased by lower concentrations of sulfuric acid. After about one hundred hours of service, a harmless black coating of nickel and chromous oxide forms on the wire. If used as indicated, the baskets should give four thousand hours or more of service.

These baskets have been used for over one year at the Rockford Health Department Laboratory, and they are still in serviceable condition.

The method here outlined appears to have the following advantages:

1. Uniform and complete cleansing, rinsing, and drying of each tube.
2. A marked saving in the cost and time required for cleaning operations.
3. Breakage of tubes is reduced to negligible quantities.
4. Reduces to a minimum the danger of acid burns to the individual cleaning tubes.
5. The cleaning solution lasts much longer.

BLOOD-CROSSING BLOCK*

E. E. MYERS, M.D., PHILIPPI, W. VA.

A TEST tube support, shown in Fig. 1, has been found very helpful in making the cross-matching of blood specimens prior to transfusion.

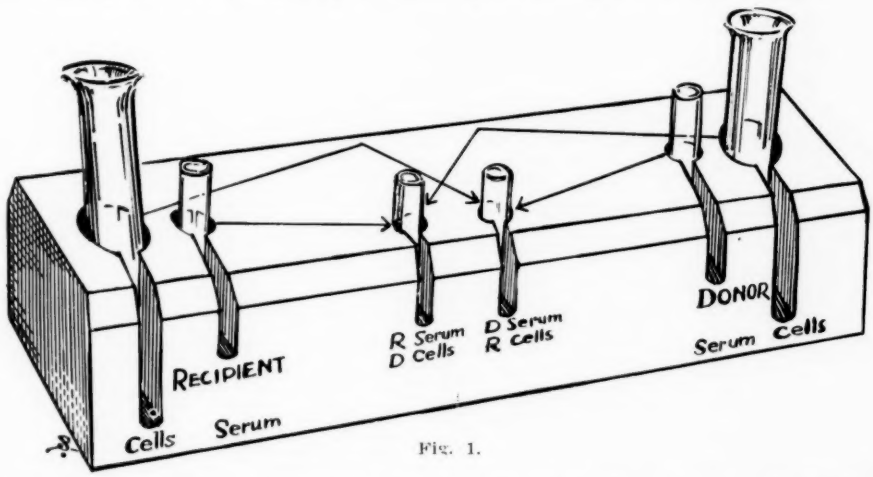

Fig. 1.

The block is of wood, painted white, size 10 by 2¼ by 1¾ inches; large holes ¾ by 1½ inches; small holes 7/16 by ⅞ inch. The middle holes have their centers ⅝ inch from the front of the block, and 1¼ inches apart. The end holes are midway between the front and back surfaces of the block, and 1 inch apart, the centers of the large holes being ¾ inch from the ends of the block. The slots are 3/16 inch wide. India ink is used to make the markings.

*From the Pepper Laboratory, University of Pennsylvania Hospital, Philadelphia.
Received for publication, October 4, 1938.

A SIMPLE DEVICE FOR DETERMINING APPROXIMATE OXYGEN CONCENTRATION IN OXYGEN TENTS*

ROBERT W. HUNTINGTON, JR., M.D.,† ST. LOUIS, MO.

A SIMPLE device used here for determining the approximate oxygen concentration in oxygen tents may merit a brief description, since the omission of such checks in many hospitals is perhaps due in part to a belief that elaborate apparatus is required. As in other bedside analyzers, oxygen is absorbed on moist copper screening with which it comes in contact as the gas sample displaces an ammonia-ammonium salt solution from the absorption chamber. Withdrawal of the sample from the chamber brings the solution back to dissolve the oxide and prepares the copper for the next analysis.

Design and Construction of the Apparatus.—The plan of this outfit is best realized from Fig. 1. A calcium chloride U-tube, with 14 cm. sides and provided with side arms and glass stopcocks, serves as absorption chamber and fluid reservoir. The top of the roll of copper gauze in the chamber side is slightly below the bottom of the cock, which is carefully lubricated with stopcock grease or a mixture of this and vaseline. The stopcock of the reservoir side is removed. The sample is measured before and after oxygen absorption in a 10 c.c. Luer syringe, graduated in 0.2 c.c. This syringe must be kept well lubricated with whatever is appropriate for the temperature. Ordinarily vaseline works best, but in very hot weather stopcock grease should be used. A small glass T-tube connects the syringe with the side arm of the chamber and with the small diameter arm of an Ayer three-way stopcock (Becton, Dickson L/S2), to the opposite arm of which is attached a rubber tube for connection with the tent or other source of gas for analysis. The T-tube should be short and of small bore, and its outside diameter should be about the same as that of the adjoining arm of the metal stopcock and the tip of the syringe, in order that these two joints may each be made with a single piece of rubber tubing. As the outside diameter of the chamber side arm will usually then be larger than that of the T-tube, it may be necessary to cover the T-tube end with fine bore rubber tubing and then use a piece with larger bore to make the connection. The glass and metal ends are brought close together, and shellac is used to make the joints airtight. To reduce the dead space between syringe, three-way stopcock, and chamber, unnecessary length in T-tube or side arm should be filed off before connections are made. Correct analytical procedure minimizes the effect of this dead space. The arrangement is advantageous, since with the glass stopcock shut one may rinse the tent connections by aspirating air from rubber tube *E* into the syringe with the metal cock in position *1*, then ejecting the air to the outside with the metal cock in position *2*. The ap-

*From the Department of Pediatrics, Washington University School of Medicine, and the St. Louis Children's Hospital.

†Theron Catlin Fellow, St. Louis Children's Hospital, 1935-38.

Received for publication, October 8, 1938.

paratus is compact enough to be mounted on a small board. The syringe, which need not be of the most expensive grade, and the Ayer stopcock are most easily obtained from a dealer in surgical supplies, while the rest of the equipment is carried by most laboratory supply houses. The total cost of the equipment is about $6.00.

Solution in the U-Tube.—In order to dissolve the copper oxide satisfactorily, it appears necessary to use an ammonia-ammonium salt solution.[1, 2] With such solutions it is difficult to exclude the possibility of volatilization of ammonia, particularly when the solution is fresh. For this reason two or three consecutive samples of room or outdoor air should be tested after renewal of the solution, and the apparatus should not be put back in service until the difference between the initial and residual samples comes to about 20 per cent. With this precaution Badger's[1] original solution of half-strength ammonia water saturated with ammonium chloride has proved quite satisfactory, and it seems to have some advantages over other solutions.[1, 4] As Badger noted, volatilization of ammonia from fresh solution, though considerable during the first determination, is usually negligible thereafter. In very hot weather, however, it has been necessary to add as much as 2 c.c. of 20° hydrochloric acid to the solution in the chamber to prevent continued liberation of ammonia gas.

The *setting of the solution* must be such that on introduction of a 10 c.c. gas sample into the chamber there will be neither spilling of solution nor escape of gas bubbles. It is convenient to have the surface of the solution in the chamber just below the top of the copper, and the layer of mineral oil covering the column in the reservoir should be between the middle and the lower end of the straight portion of the tube. This difference in the height of the two columns is induced by withdrawing air from the chamber into the syringe while the glass stopcock is open and the metal cock is in position *3*, which, connecting tube *E* with the outside, shuts off both from the T-tube. After the levels are set, the glass stopcock may be closed and the syringe emptied of air with the metal cock in position *2*.

To maintain this setting, the glass stopcock must not be opened when the metal cock is in position *1* or *2*. If such an accident occurs, the glass cock is shut, and any gas in the syringe is ejected to the outside with the metal cock in position *2*. The metal cock is then turned to position *3*, the glass stopcock is opened, and the plunger is moved back and forth several times to allow complete absorption of the oxygen in the system. The levels are then reset.

Oxygen Determinations.—The procedure differs but little from that of other bedside apparatus. The rubber tube is connected with the tent outlet valve or introduced under the hood. A small gap in the zipper of one of the newer hoods makes possible an almost airtight connection. After repeated rinsing of the tube by the procedure already described, the sample for analysis is taken by withdrawing the plunger precisely to the 10 c.c. mark. The plunger is held at this mark while the metal cock is turned from position *1* to position *3*, and the glass stopcock is opened for an instant, then shut. A shift of the oil meniscus greater than the equivalent of about 0.2 c.c. indicates that the analysis should be abandoned, but such shifts have been obtained only when the sample had been taken from a source in which the pressure was markedly greater or less than atmos-

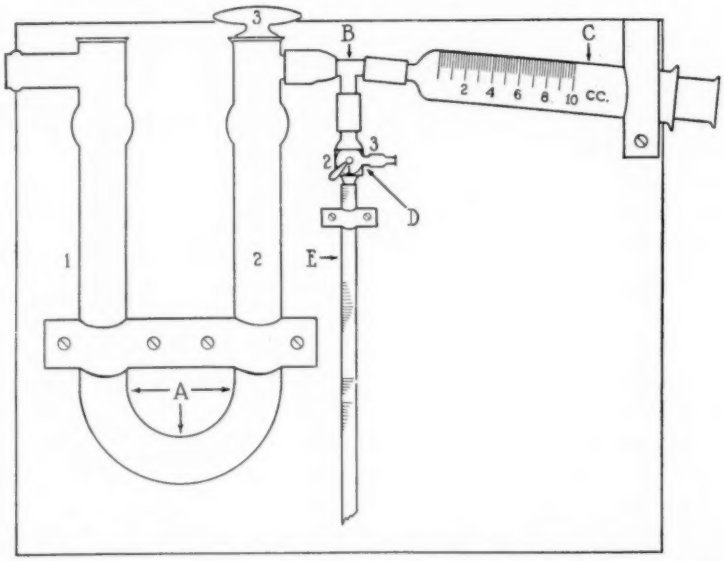

Fig. 1.—*A*, U-tube; *1*, reservoir side; *2*, absorption chamber side (contains copper gauze); *3*, handle of glass cock. *B*, T-tube. *C*, Syringe. *D*, Three-way metal stopcock; *1*, setting of handle for position No. 1; *2*, setting for position No. 2; *3*, setting for position No. 3. *E*, Rubber tubing from tent.

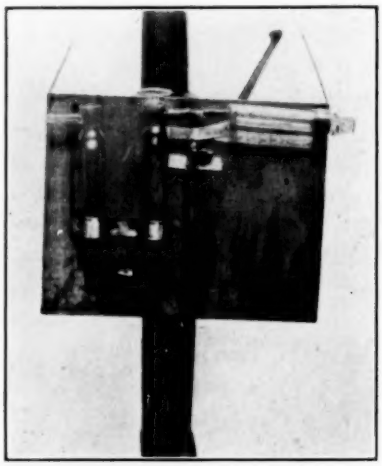

Fig. 2.

pheric, never with tent samples. With the not infrequent slight shifts the thread tied around the reservoir tube is simply adjusted from the old to the new level of the oil meniscus. The glass stopcock is again opened as the plunger is pushed all the way in, then promptly shut. This stopcock is not reopened until just before the final reading. Since the rinsing has made the gas in the syringe and T-connection practically homogenous, the air displaced from the T-connection to the chamber as the plunger is first pushed in will be equivalent to the air left in the T-connection when the syringe is emptied. Since this air is not allowed to lose its oxygen, the assumption that deoxygenation affects neither more nor less than a valid 10 c.c. sample involves no significant error. As a matter of fact, the

dead space in our outfits was so small in comparison with the volume of the syringe that repeated moving of air to and from the chamber made only a slight difference in the result. After three minutes have been allowed for absorption, if the fluid line on the copper has stopped climbing, the glass stopcock is opened and the plunger withdrawn until the oil meniscus is brought down to the thread which marked its position before the sample was transferred to the chamber. The position of the plunger now marks the final reading. The calculation: 10 (10 minus final reading in c.c.) = percentage of oxygen. If desired, a per cent scale may be marked on a strip of adhesive tape beside the syringe. After the reading, the top of the copper may be washed off by withdrawing the plunger a little further. It is best not to bring the solution quite up to the bottom of the glass cock, for lubricant, if picked up repeatedly by the solution, might eventually coat much of the copper and hinder oxygen absorption. The solution is reset, the glass stopcock is closed, and the syringe emptied with the metal cock in position *2*.

Care of the Apparatus.—A year's experience has shown that these outfits are sturdy and require little attention. When the solution begins to show a green precipitate, it should be poured out of the reservoir side after removal of the chamber cock. Occasionally a considerable amount of oil or grease will have accumulated in the chamber. Such material may be floated out by washing the U-tube through and through with water. Fresh Badger's solution is poured in through the reservoir side, and the chamber stopcock is dried and lubricated. The column of fluid in the reservoir is covered with fresh mineral oil, and the levels are reset. The syringe and the glass stopcock must be kept lubricated, and occasional aspiration of a little mineral oil through the metal stopcock may be helpful. The Badger's solution should not be allowed to reach the chamber side arm and its connections. Leaks should be searched for occasionally by introducing a little air into the syringe, then turning the metal cock to position *3*, and with the glass stopcock closed, first pulling, then gently pushing, on the plunger. If the fluid in the U-tube does not shift, and the plunger moves back to approximately its previous position on release, a further test may be performed by letting rubber tube *E* hang down into a beaker of water and aspirating water into the syringe, air being eliminated from the connections by the usual rinsing procedure.

Accuracy of the Determinations.—This is limited by the fact that neither the oil meniscus nor the syringe is adapted to precise reading. The error of reading may amount to as much as 2 or 3 per cent oxygen.

Reliability of these determinations within this limit was demonstrated both by analyses of outdoor air and oxygen from a tank and by parallel determinations of tent air with the simple outfit and with the Haldane. The former procedure is the simpler for routine checks. The pure oxygen is most easily obtained through a T-tube connection which permits release of excess pressure.

For Haldane analysis tents were used which permitted connection of a rubber tube either through an outlet valve or through a tight-fitting gap in a zipper in order that change in the tent air might be minimal during sampling. The specimen for the Haldane was taken with a Collins sampling pump, while the sample for the bedside analyzer could be obtained either from the pump or directly from the tent, provided that in the first case the pump pressure was kept

close to atmospheric pressure during transfer of the sample, and in the second case the end of the rubber tube *E* was placed in the main stream of circulating tent air. Determinations with the bedside analyzer were always made first to determine the gas volume which must be handled in the Haldane in order to have a residual large enough to be measured in the graduated portion of the burette. This volume was always more than 10 c.c., since the oxygen content of the samples was 40 to 65 per cent. It was necessary, therefore, to introduce two or more consecutive samples into the burette. After the first sample was measured, it was promptly transferred in toto to the pyrogallol chamber, the mercury being brought precisely to the top of the burette by adjustment of a pinchcock. The carbon dioxide content of the specimen was then determined on the second sample. After the burette was again connected with the pyrogallol chamber, the leveling bulb was cautiously raised and lowered several times until the gas volume had shrunk so that spilling of pyrogallol or escape of bubbles need no longer be feared. Absorption was then completed in the usual manner with at least two rinsings of the air left in the hydroxide capillaries. Although not capable of the highest precision, this method gave oxygen values within 0.1 per cent of one another on duplicate analysis.

Limits to precision attainable with any bedside apparatus are such that determinations with the outfit just described are perhaps as accurate as those obtainable with most others. With all of them the directions call for round number measurement of the initial volume. The setting at which the final reading is to be made is usually that assumed by the ammoniacal solution, as connection is made between the chamber and the sample container (syringe or burette) while the plunger or meniscus of the latter is held at the round figure. Precision demands that as in the Haldane, initial as well as final volume be measured in accordance with a previous setting, and such a measurement does not usually come in round numbers. If the initial and final readings are made at approximately the same pressure, the difference will represent well enough the volume which the absorbed gas had occupied at that pressure, but the original pressure adjustment between chamber and sample container, if appreciable, will invalidate the initial reading. Another source of uncertainty could only be eliminated by separate determination of carbon dioxide, for the copper-ammonia chamber, like other devices for oxygen absorption, absorbs carbon dioxide as well. Thus it was found that two successive samples of 70 per cent oxygen and 30 per cent carbon dioxide were almost completely absorbed in our apparatus. Absorption of several consecutive samples of approximately pure carbon dioxide was likewise complete, even when Badger's solution was replaced by saturated aqueous ammonium chloride. This observation illustrates the well-known ability of moist copper to absorb carbon dioxide[2] and shows that such absorption in an ordinary copper-ammonia chamber need not be attributed solely to the alkaline solution. The use of ammonium carbonate solutions offers no way out of this difficulty, since they tend to give off both carbon dioxide and ammonia, and to inhibit the absorption of oxygen.[1] A third possibility of error, the volatilization of ammonia already mentioned, makes the copper-ammonia chamber unsuitable for research purposes. However, such chambers, with their safety, rapid action, and prolonged usefulness, are not likely to be displaced from bedside work by either pyrogallol or hyposulfite.

Comparison with other analyzers suggests that those of Hochstetter[3] and Riesman and Lesnick[4] are slightly more complicated in construction than the one here described. They are equipped with finer scales for measurement of the residual sample, and may be even simpler in operation. The author's prejudices in favor of his own device are based on the readily rinsed connections, the use of the syringe for all measurements, which obviates the necessity of a second scale, the ease with which solution or even copper may be renewed, the compactness of the mounting, and the simple construction which requires neither cement nor glass blowing. If others should share these views, this apparatus might attain wider use.

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A SIMPLE TECHNIQUE FOR CONCENTRATING TUBERCLE BACILLI IN SPUTUM*

N. P. SULLIVAN, M.S., AND H. J. SEARS, PH.D., PORTLAND, ORE.

ONE hesitates to add a technique for sputum concentration to a literature already oversupplied with methods and modifications. For such a new method to be worth while it should be (1) less expensive, (2) more rapid, (3) more simple in manipulation, or (4) give better concentration of the tubercle bacilli than methods now in use. We believe that the method to be described fulfills some or all of these requirements and thereby justifies its presentation.

The digestant used in our method is a crude preparation of the enzyme papain which is marketed commercially under the name Caroid by the American Ferment Co., Buffalo, N. Y. Other preparations of papain are on the market and might be quite as efficient. We have not tested any of them.

The method for using the papain digestant is as follows: Approximately 0.5 gm. of papain, either in the powder form or mixed with a small amount of water to form a paste, is mixed with 50 c.c. of sputum. The proportions of enzyme to sputum may be varied considerably without affecting the results. If a large number of sputa are to be concentrated at once, it is more convenient to dilute the enzyme with water just before adding it to the sputa. The stock enzyme, however, should always be kept in the powdered form as the solutions do not keep well.

*From the Department of Bacteriology, University of Oregon Medical School, Portland.
Received for publication, October 10, 1938.

The enzyme-sputum mixture is incubated at 37° C. in either a water bath or hot-air incubator. As soon as complete liquefaction occurs, the mixture is centrifuged at high speed, and smears are made from the sediment and stained in the usual way. The time required for liquefaction varies with the sputum and the enzyme concentration. The average time is ten to fifteen minutes. Papain digestion gives a creamy sticky sediment that adheres well to slides. Because of this, much thicker smears are possible than by the usual concentration methods. Cellular elements and organisms other than the tubercle bacilli are not destroyed by this method. Because other organisms are not destroyed, if cultures or animal inoculations are to be made further treatment of the sediment is necessary. We found that treating the sediment with either acid or alkali gave satisfactory cultures and animal infections.

Cultures of the powdered enzyme showed it to contain a number of various kinds of organisms. For this reason each batch of enzyme was tested for acid-fast bacilli. In no case were any acid-fast organisms found.

To test the efficiency of the enzyme digestion, 50 sputa were selected that contained from one organism in 10 fields to 10 organisms per field, as determined by direct smear. Each sputum was then divided into equal parts, one-half was digested by 4 per cent sodium hydroxide, the other by the enzyme method. After digestion direct smears were made from the enzyme-digestion mixture before centrifuging to compare these smears with those made directly from the undigested sputum. Smears of the sediment from the enzyme and the sodium hydroxide concentrations were made and all were stained by the Ziehl-Neelsen method. In addition to the methylene blue counterstain duplicate slides were counterstained with a saturated aqueous picric acid solution. Extra thick smears were also tried from both types of concentrated sediment. In the case of the thick smears the picric acid counterstain was by far the better. In addition to centrifuging, chemical flocculation, as advocated by Hanks, Clark, and Feldman,[1] was used on the enzyme-digested sputum. It was found that chemical flocculation with either ferric chloride or potassium alum could be used on enzyme-digested as well as with alkali-digested material.

It was found that sputum that had been enzyme digested, well mixed and smeared, gave a higher average number of organisms per field and a much more even distribution of organisms than by direct smear from undigested sputum. In many cases the smears from the digest gave twice as many organisms per field as did the direct smears. In the case of the centrifuged enzyme-digested sputum, the number of organisms per field was practically the same as by the alkali digested in 90 per cent of the sputa. In 10 per cent the enzyme digestion method was definitely superior. Following are a few illustrative counts:

	Number of Organisms per Field in Duplicate Specimens				
Papain digestion	101	79	99	40	55
Sodium hydroxide digestion	90	58	83	31	42

Cultivation and Animal Inoculation of Material Digested by Papain.—Twelve sputa were selected that were positive for acid-fast bacilli in enzyme-digested sediment. The sediments were treated with either acid or alkali. Cultures were made on Corper's and Petroff's media and guinea pigs were inocu-

lated. Positive cultures were obtained from all of the sputa, and autopsies of the guinea pigs showed many typical lesions in all of the animals.

Effect of Enzyme Digestion on Organisms Other Than Tubercle Bacilli.—The sediments from all of the enzyme-digested sputa were stained by Gram's method and by Churchman's capsule stain. In all cases the Gram stain appeared typical, and in a few specimens encapsulated organisms were demonstrated by the Churchman method. A number of organisms in pure culture were then submitted to the action of the enzyme. *Hemolytic streptococcus, E. typhi, S. paratyphi, Esch. coli, D. pneumoniae, C. diphtheriae, and C. hofmanni* strains were grown on Douglas chocolate agar for twenty-four hours, the growth emulsified in 5 c.c. of saline papain solution and incubated for three hours at 37° C. Streaks were then made from these on Douglas blood agar and Douglas chocolate agar plates. In every case the specified organism was recovered. As stated previously, the enzyme powder is not sterile, so the streak plates contained organisms other than those under test. No difficulty was encountered, however, in isolating the test organism.

CONCLUSIONS

An enzyme-digestion method for concentrating the tubercle bacilli in sputum has been described which has the following advantages.

1. Digestion of sputum is rapid, usually being complete within ten to fifteen minutes.

2. No neutralization of the digestant is required.

3. The sediment obtained from the enzyme digestion adheres well to glass slides, allowing thicker smears.

4. Cultures and animal inoculations may be made from the sediment after appropriate treatment to destroy nonacid-fast organisms, which are not affected by the digestant.

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A MANUAL OF NEUROHISTOLOGIC TECHNIQUE

Oscar A. Turner, M.D., New Haven, Conn.

(Continued from the June issue, page 1003.)

Chapter XI

Miscellaneous Staining Methods

Iron.—There are many methods for the microchemical determination of iron in tissues. A simple and rapid procedure is the Perl test which depends upon the formation of a blue color by the action of potassium ferrocyanide upon the iron contained in hemosiderin. The method is sometimes referred to as the Prussian blue reaction.

1. Sections are brought to distilled water. Frozen or celloidin sections may be used, but in the latter the celloidin must be removed before staining.

2. Treat for thirty minutes to one hour in a mixture of 1 part freshly prepared 2 per cent potassium ferrocyanide and 3 parts of 1 per cent hydrochloric acid by weight. The latter is prepared by adding 2 c.c. of concentrated hydrochloric acid to 75 c.c. of distilled water.

3. Wash thoroughly in four to five changes of distilled water.

4. Counterstain lightly with eosin.

5. Dehydrate in alcohols, clear in xylol, and mount in balsam. The iron (hemosiderin) is stained blue upon a pink or red background. The stain is quite intense and very sharp. The best results are obtained upon alcohol or formalin-hardened tissue.

Zenker-fixed tissue may be used, but the blue color is apt to be diffuse. Paraffin, celloidin, or frozen sections may be used. Mallory recommends counterstaining with 0.1 to 0.5 per cent basic fuchsin in 50 per cent alcohol. With this counterstain the nuclei and hemofuscin are stained red, and the hemosiderin blue. The preparations tend to fade in several months.

For the rapid detection of iron in brain tissue, especially in suspected cases of dementia paralytica, the Spatz reaction is useful. Material from the frontal cortex will give the most pronounced results. A small piece of fresh, unfixed cortex is immersed for a short time in physiologic saline solution. It is then transferred to concentrated ammonium sulfide and allowed to remain fifteen minutes. In general paralysis one sees fine, dark streaks and punctate areas in the cortex. By means of a glass hook such an area is removed and brought to physiologic saline. It is then trimmed of as much excess tissue

as possible and transferred to a small drop of glycerin on a glass slide. A cover slip is placed over it, and by firm pressure the tissue is spread flat. Under the microscope one can distinguish greenish-black clumps and granules of iron in the vessel walls and cells.

Calcium.—Calcific deposits in tissue stain deeply with hematoxylin so that special methods are usually not necessary. Von Kossa's method is useful when a differential stain for these lime deposits is needed.

1. Paraffin, celloidin, or frozen sections of alcohol or formalin-hardened tissue are used.
2. Place sections in a 1 to 5 per cent solution of silver nitrate for thirty to sixty minutes.
3. Wash thoroughly in distilled water.
4. Dehydrate in ascending alcohols, clear in xylol, and mount in balsam.

Calcium salts are stained a deep black. The nuclei can be counterstained with alum carmine or safranine after the silver impregnation. The method depends upon the formation of silver carbonate or silver phosphate which, in the presence of light, is converted to black silver oxide. If the sections are left in the silver solution as long as twelve hours, there is more assurance that all of the calcium phosphate will have been stained.

The sections should be exposed to direct sunlight while in the silver bath. The reaction may be hastened by a few minutes' exposure to a direct source of ultraviolet light.

Staining Tumors for Rapid Diagnosis.—It is often desirable to obtain a quick histopathologic report upon neoplasms exposed during an operation. The fact that many of the intracranial tumors are ill-suited for frozen-section procedures makes desirable some method whereby unfixed bits of tissue may be stained rapidly and prepared for microscopic examination.

Supravital Staining.—This method has been applied by Eisenhardt and Cushing with good results. The method has the disadvantage of requiring familiarity with the appearance of cells stained with a dilute solution of neutral red dye.

As soon as the tumor is exposed at operation a minute fragment is separated and placed on a clean glass slide. A drop of aqueous solution of neutral red dye (1:10,000 or more) is added directly to the tissue. A cover slip is then applied, and by gentle pressure the tissue is spread. The preparation is ringed with a paraffin-vaseline mixture and examined immediately under the microscope in a warm box.

The amount of pressure required to obtain an even preparation varies with the type of tissue and is learned only by experience. If the solution of the dye is too strong, the nuclei will stain diffusely, a sign that the cells have been injured, or the tissue will be overstained, making the diagnosis difficult. It is important that the preparation be made as soon as possible after the tissue has been removed. Tissue which has been allowed to dry is useless, but stained preparations retain their clarity for an hour or more if kept in the

warm box and if drying is prevented. Upon standing there is a tendency for the cells to become vacuolated and for the cytoplasm to become granular.

The microscopic appearance of each of the various types of intracranial tumors when stained in this manner can be found in the original articles by Eisenhardt and Cushing. However, a few characteristics of the more common types will be given. The fibrillary astrocytomas usually appear as a dense network of neuroglia fibers, which are rarely stained but which appear as highly refractile processes with scattered nuclei. In the less dense areas along the margins of the preparation an unmistakable astrocyte with long processes may be seen. Occasionally there is an abundance of astrocytes. In tumors which are composed predominantly of the protoplasmic astrocytes there are few fibrillae and, if the specimen is not too degenerate, astrocytic cells may be seen. Tissue from glioblastomas shows cells of various shapes and forms, astrocytes, spongioblasts, and multinucleated forms. There may be many mitotic figures. In some instances, if the tissue is necrotic, the field may consist of large, vacuolated cells filled with refractile globules having a characteristic greenish-red color. Medulloblastomas appear as closely packed round cells with pale but well-defined cytoplasm. Mitotic figures are abundant. The cells of the oligodendrogliomas are also well defined. The nuclei are spherical, contain considerable chromatin, and are surrounded by an abundance of pale cytoplasm. Mitotic figures are not seen. The meningiomas are easily recognized by the characteristic whorl formation. The cells have large oval nuclei and are usually well defined.

It should be noted that if nervous tissue from the immediate region of a tumor is examined, an unusual number of neuroglia fibrillae and astrocytes may be observed. A representative piece of tissue is, therefore, necessary for diagnosis. The recognition of characteristic myelin sheaths will often differentiate a reactive gliosis from actual tumor tissue.

AzureA-Erie Garnet B Stain.—This method, described by Deery, has been found useful for rapid diagnosis and requires no special experience.

A small piece of tumor, trimmed free from all blood clot and apparent non-neoplastic tissue, is transferred to a new, clean glass slide. With two dissecting needles the tissue is teased into small fragments or a thin layer, depending upon its consistency. A few drops of the dye mixture of azure A and Erie garnet B are then added, and the preparation is allowed to stand about fifteen seconds. Without washing off the excess stain a cover glass is applied and the preparation is firmly pressed between two blotters. It is then examined under the microscope.

The preparation of the staining solution has been described by Geschickter. To 4 parts of a previously filtered 1 per cent aqueous solution of azure A, there is added 1 part of a filtered 0.5 per cent aqueous solution of Erie garnet B. The latter is added quickly and the resultant mixture is filtered immediately to prevent precipitation. The mixture keeps well, but it may be necessary to refilter after it has been standing for several weeks.

For frozen sections Geschickter recommends staining for ten to fifteen minutes in the dye mixture, washing in two changes of distilled water and mounting in 40 per cent glucose. After the second wash, the section is trans-

ferred to a clean slide where a cover slip having upon it a large drop of 40 per cent glucose is inverted over the section. The preparation may be examined after allowing a minute or so for clearing to take place. The section should be handled with a glass hook from which it need not be removed until ready for mounting.

The azureA-Erie garnet B method gives a stain which is similar to hematoxylin and eosin, but the final preparation is said to be much clearer and with greater detailed staining. The stain does not fade.

Stains for Negri Bodies.—Only occasionally in the average laboratory is there need for a stain for Negri bodies. It is advisable, therefore, that the methods employed use staining solutions which are either quite stable or readily prepared. The tissue examined should be from either the cornu ammonis (hippocampus major) or cerebellum, and if smears or impressions are to be prepared, fresh, unfixed tissue is necessary. If the tissue is to be embedded in paraffin, it should be fixed in alcohol or acetone as soon after death as possible. To prepare an impression, place a small disk of tissue upon a board with one of the cut surfaces up. Press the surface of a clean glass slide upon the disk of tissue and lift it suddenly. If the tissue has not been allowed to dry, it will adhere to the board and leave an impression on the slide. To prepare a smear, place a small piece of tissue on a clean slide. With another slide used as a spreader, press evenly and firmly upon the tissue until it covers the width of the slide. Then draw the spreader toward the opposite end of the glass slide, maintaining an even pressure. The piece of tissue should not be too large, otherwise a thick smear will result.

Sellers' Method.—This method has found wide usage but has the disadvantage of requiring undried and unfixed smears or impressions.

The preparations, while still moist with the tissue juices, are plunged into the staining solution and immediately removed. They are then washed in tap water and allowed to dry in the air. The staining solution is prepared in the following manner:

Basic fuchsin (saturated absolute methyl alcoholic solution)	2 to 4 c.c.
Methylene blue (saturated absolute methyl alcoholic solution)	15 c.c.
Methyl alcohol (absolute, acetone free)	25 c.c.

The methylene blue solution and methyl alcohol are mixed in a Coplin jar and 2 c.c. of the basic fuchsin solution are added. A trial smear is made. Macroscopically, the properly stained smear should appear reddish violet in the thinner portions. If on trial smears the thinner portions are blue, more of the basic fuchsin is added. Two cubic centimeters are all that is usually required. The mixture improves after standing for twenty-four hours, and keeps indefinitely if evaporation is made up with additional methyl alcohol. Evaporation causes the fuchsin stain to become dominant. The staining solution should be kept in a tightly stoppered bottle.

The Negri bodies are stained a bright, cherry red, and the basophilic or blue staining masses are quite clear. The cytoplasm of the nerve cells is a

purplish blue, the nuclei a deep blue. Nerve fibers are stained a deep pink and bacteria, if present, an intense blue. Erythrocytes appear copper colored. Myelin sheaths are not stained.

While the best results are obtained from fresh tissue, the Negri bodies often may be recognized in degenerated areas. The method is useful for smears of pus and exudate and stains many forms of protozoa, especially the flagellates and ciliates. However, smears which have been allowed to dry or which have been fixed in alcohol, formol, or Zenker's solution, or any other fixative cannot be used. Sections of tissue cannot be stained by this method. It is important that the methyl alcohol be absolutely free from xylol and that the smear be moist when plunged into the stain. Staining should not exceed a few seconds, since even after thirty seconds the results are practically useless.

Mann-Böhne Method.—The method is applicable to smears or sections. The tissue should be fixed in alcohol or acetone and embedded rapidly in paraffin.

1. Stain thin sections for one-half to four minutes in the following mixture:

1% aqueous methylene blue	35 c.c.
1% aqueous eosin	35 c.c.
Distilled water	100 c.c.

2. Wash briefly in water.

3. Wash in equal parts of absolute alcohol and water.

4. Allow sections to remain for fifteen to thirty seconds in alkaline alcohol. This is prepared by the addition of 5 drops of 1 per cent sodium hydroxide in absolute alcohol to 30 c.c. of absolute alcohol.

5. Wash briefly in absolute alcohol.

6. Wash in tap water.

7. Transfer to tap water which has been made acid by the addition of 1 drop of acetic acid to 30 c.c. of water. Allow to remain one to two minutes.

8. Wash rapidly in absolute alcohol and clear in xylol. Mount in balsam.

This is an excellent stain provided the sections are not overdecolorized in the alkaline alcohol. The Negri bodies are seen as small oval bodies stained a deep pink, lying within the pale blue cytoplasm of the nerve cells. There is often a more deeply stained peripheral zone with one or more small vacuole-like bodies within it.

In addition to the above two methods, excellent preparations may be obtained upon embedded material by the method of Lentz, or upon smears or impressions by the methods of Frothingham, van Gieson, and Giemsa.

Gross Staining of the Brain.—Practical museum preparations, especially useful for the teaching of anatomy or pathology, may be obtained by the macroscopic staining of sections of the brain. Such preparations, when properly stained, give sharp contrast between gray and white matter and can be

kept indefinitely for display or class use. The following method, described by Le Masurier, has been found to be the most dependable.

1. The brain should be thoroughly hardened in formalin. Slices are cut to the desired thickness with a knife which is well lubricated with glycerin. The sections must be cut in one stroke to avoid leaving knife marks. They should not be handled with the fingers.

2. Wash the specimens from twelve to twenty-four hours in running water. Transfer to distilled water and allow to remain for one hour, changing the water at least three times during this period.

3. Submerge the sections for two minutes in Mulligan's solution of phenol at from 60° to 65° C. There should be enough of this solution to cover the sections by 2 to 3 inches. The formula for Mulligan's solution is as follows:

Phenol crystals	40.00 gm.
Concentrated hydrochloric acid	1.25 c.c.
Copper sulfate (crystals)	5.00 gm.
Distilled water	1000 c.c.

4. Submerge in an abundance of cold tap water for one minute.

5. Transfer to a 1 per cent solution of ferric chloride in distilled water and allow to remain two minutes.

6. Place in a 1 per cent aqueous solution of potassium ferrocyanide until the gray matter is a brilliant blue. This should take no longer than three minutes.

7. Wash in running water for twenty-four hours.

8. Preserve sections in 70 per cent alcohol.

The cortex and central nuclei, such as the caudate, thalamus, and dentate of the cerebellum, are stained a brilliant blue. Areas of demyelinization and degeneration are also stained blue as are intracranial tumors. The white matter remains a creamy white. The preparations may be exposed to direct sunlight without fading. It is very important that the knife used to cut the brain be well lubricated with glycerin and that the slice be cut in one stroke. Failure in this respect will result in diffuse coloration of the white matter. Sections should be handled as little as possible and then with great care. Excessive and rough handling of the slices will cause the white matter to assume a blue color.

Histotopographic Methods.—Largely through the work of Christeller, a technique has been devised for the preparation of frozen sections of complete organs. These preparations are suitable for microscopic study. The method is of particular value in that it allows for both a topographic and a microscopic survey of a cross section of a complete organ. The microtome used for the purpose of making these large frozen sections is of a special type with a large freezing chamber and double fixation of the knife to prevent vibration. For the exact technique the reader is referred to the Foreword in Dr. Christeller's Atlas or to the article by Dr. Maude E. Abbott.

Chapter XII

Formulas

Zenker's Fixative.

Potassium bichromate	2.5 gm.
Mercuric chloride	5.0 gm.
Water	100.0 c.c.
Glacial acetic acid	5.0 c.c.

Dissolve the potassium bichromate and mercuric chloride in the water with the aid of heat. Add the glacial acetic acid in the proper proportion just before use. Tissue should be fixed in this fluid for ten to twelve hours. Prolonged fixation produces crystals of mercuric chloride which are difficult to remove. Sections of tissue which has been fixed in Zenker's fluid must be treated with Gram's iodine and 5 per cent sodium thiosulfate before staining to remove the crystals which are deposited.

Regaud's Fixative.

5% potassium bichromate	3 parts
Commercial formalin	1 part

The formalin is neutralized with magnesium carbonate. The solution should be changed daily. This is the best fixative for pituitary tissue and for certain of the nonmetallic glial stains.

Orth's Fixative.

Potassium bichromate	2.5 gm.
Water	100.0 c.c.
40% formalin	10.0 c.c.

This is a good general fixative. It is improved by the addition of 5 per cent acetic acid just before use.

Müller's Fluid.

Potassium bichromate	2.5 gm.
Sodium sulfate	1.0 gm.
Distilled water	100.0 c.c.
Heat together until dissolved.	

This is a slowly acting fixative valuable for nervous tissue. The solution should be changed frequently and kept in the dark to prevent precipitation.

Cajal's Formalin-Ammonium Bromide.

Formalin (Merck's blue label, 40%)	14. c.c.
Ammonium bromide	2 gm.
Distilled water	86 c.c

This fixative is used particularly for the demonstration of the interstitial cells of the nervous system by the metallic impregnation methods. Tissue should not remain in this solution for more than six to seven days.

Formol Saline.

Sodium chloride	1 gm.
Formalin	10 c.c.
Water, to make	100 c.c.

This can be used as a routine fixative in place of 10 per cent formalin. Addison, in McClung's *Handbook*, recommends the addition of sufficient sodium chloride to 5 per cent formalin solution to bring the specific gravity to 1.030. He states that in this solution there is less initial change and that the brain floats, submerged in the solution, thus preventing distortion by the sides of the container.

Kaiser's Glycerin Jelly.

Finest French gelatin	8 gm.
Phenol crystals	5 gm.
Glycerin	50 c.c.
Distilled water	42 c.c.

Mix the glycerin and gelatin together and place in a water bath at 55° C. until the gelatin is completely dissolved. Add the phenol crystals and filter. Allow to cool and harden. Melt in a bath of boiling water before use. The gelatin may be dissolved first in the water, and the phenol and glycerin added later. The solution must be filtered through glass wool. If coarse filter paper is used, the process must be carried out in an incubator and requires eighteen to twenty-four hours.

Arabic Sugar.

Pure gum arabic	20 gm.
Sucrose	20 gm.
Distilled water	50 c.c.

Dissolve the sugar in the water. Rub the gum arabic until finely pulverized and add slowly, avoiding the formation of bubbles as much as possible. Add a few crystals of phenol and stopper tightly. Heat in an oven until thoroughly dissolved. The solution should be clear. This medium has a refractive index the same as balsam. It sets quite hard and can be used where sections must be mounted directly from water.

Delafield's Hematoxylin.

Hematoxylin crystals	4 gm.
Absolute alcohol	25 c.c.
90% alcohol	100 c.c.
Glycerin	100 c.c.
Saturated aqueous solution of ammonia alum in distilled water	400 c.c.

Dissolve the hematoxylin in the absolute alcohol and add the saturated ammonia alum solution. Mix well and allow to stand open, exposed to direct sunlight if possible, for three to four days. Filter and add the glycerin and 90 per cent alcohol. The mixture should be diluted considerably with distilled water before using. About 30 to 40 gm. of ammonia alum are required

to saturate 400 c.c. of water. This is a powerful nuclear stain which keeps well. It may be necessary to ripen the solution for a long period to obtain the optimum results.

Ehrlich's Hematoxylin.

Hematoxylin crystals	2 gm.
96% alcohol	100 c.c.
Distilled water	100 c.c.
Pure glycerin	100 c.c.
Potassium alum	3 gm.
Glacial acetic acid	10 c.c.

Dissolve the hematoxylin in the alcohol and add the other ingredients, leaving the acetic acid to the last. The solution should be a light red when first mixed, but should darken on exposure to light. It should be placed in a cotton-stoppered glass flask and exposed to direct sunlight for at least fourteen days. Better results are obtained after exposure to light for six months. About 0.5 gm. of hematin may be used instead of the hematoxylin, in which case the solution may be used sooner. The nuclei stain a clear blue. After staining the section should be washed thoroughly.

Mayer's Hematoxylin (Hemalum).

Hematoxylin	1.0 gm.
Distilled water	1000.0 c.c.
Sodium iodide	0.2 gm.
Potassium alum	50.0 gm.

This is an improvement over the original Mayer formula. The hematoxylin may be first added to 50 c.c. of 90 per cent alcohol and dissolved with the aid of heat. The water and alum are then added. The solution acts much as Delafield's hematoxylin, but does not keep as well. A crystal of thymol may be added as a preservative. The addition of 2 per cent glacial acetic acid to this stain constitutes Mayer's acid alum. The acid preparation gives a clearer nuclear stain than does the above formula.

Weigert's Iron Hematoxylin.

A. Hematoxylin	1.0 gm.
96% alcohol	100.0 c.c.
B. Ferric chloride	0.4 gm.
Distilled water	100.0 c.c.
Hydrochloric acid	1.0 c.c.

Equal parts of these two solutions are mixed just before staining. Although the mixture keeps for some time, it is best to prepare it fresh. Decolorization may be carried out in a weak solution of ferric chloride.

In certain staining methods, especially those in which strongly acid or alkaline solutions or prolonged treatment in silver salts is used, there may be a tendency for paraffin sections which have been mounted by the glycerin-egg albumen method to float off the slide. If possible, the sections should be mounted with gelatin. However, for routine purposes, the following method

has been useful in preventing detachment. The unstained sections, following treatment with several changes of xylol and hydration through descending alcohols, are brought to water and then to a Coplin jar containing 1 to 3 per cent gelatin solution. They are allowed to remain here at least four hours and as long as twelve hours. Following this they are transferred to 10 per cent formalin without washing and allowed to remain for a similar length of time. Staining is carried out as usual following washing in tap and then distilled water. Provided the sections are not handled roughly, they will adhere to the glass slides.

Sections which are treated with the Weigert mordants before staining should be passed through the gelatin and formalin as outlined above. The method does not interfere with the staining properties of the tissue.

Appendix

A. *Nitrocellulose as an Embedding Agent.*—Nitrocellulose may be used as an embedding agent in place of the more expensive celloidin. Since this agent is an explosive when dry, it is usually supplied thoroughly moistened with alcohol. The alcohol content of the material must be taken into account in the preparation of the solution.

The tissue to be embedded, after having been dehydrated in alcohols of ascending strengths, is passed through alcohol-ether solution (1:1) and allowed to remain for a period of time in 6 per cent and finally 12 per cent nitrocellulose. The length of time required for complete infiltration of the tissue to take place varies with the size of the tissue block and the concentration of the nitrocellulose solution and must be gauged accordingly. After the tissue has been thoroughly infiltrated, it is embedded in fresh 12 per cent solution. However, it may also be allowed to stand for some time in 25 per cent nitrocellulose and embedded in this solution. The final method of hardening the block and sectioning does not differ from that used with celloidin. As with celloidin, the hardening should take place slowly and should not be carried to an extreme.

The following table gives the proportion of the ingredients used in the preparation of approximately 560 c.c. of nitrocellulose solution:

	25% Solution	*12% Solution*	*6% Solution*
Nitrocellulose	200 gm.	100 gm.	50 gm.
Absolute alcohol	205 c.c.	245 c.c.	260 c.c.
Ether	280 c.c.	280 c.c.	280 c.c.

The 25 per cent solution may give rise to difficulties in embedding because of entrapped air bubbles. The 12 per cent solution may be prepared by diluting the 25 per cent solution with an equal part of absolute alcohol-ether solution. Similarly, the 6 per cent solution may be prepared by diluting the 25 per cent solution with 3 parts of absolute alcohol-ether solution or the 12 per cent solution with an equal part of absolute alcohol-ether solution. The 6 per cent and 12 per cent solutions may also be prepared from the dried and finely chopped

scraps of nitrocellulose obtained in trimming the embedded blocks. The proportions used here are as for any solution.

Although the time required to embed tissue is slightly longer than with celloidin, the results are equally as good and far less expensive.

B. *Dye Solubilities.*—The following table lists the solubilities for various dyes in both distilled water and 95 per cent alcohol, as given by the Stain Commission. The figures represent the number of grams of stain in 100 c.c. of a saturated solution at 26° C.

Dye	*Water*	*95% Alcohol*
Picric acid	1.18	8.96
Orange G	10.86	0.22
Safranine	5.45	3.41
Cresyl violet	0.38	0.25
Thionine	0.25	0.25
Toluidine blue	3.82	0.57
Sudan III	0.00	0.15
Sudan IV (scharlach R)	0.00	0.09
Crystal violet, chloride	1.68	13.87
Eosin, sodium salt	44.20	2.18
Eosin B	39.11	0.75
Erythrosin	11.10	1.87
Methylene blue, chloride	3.55	1.48
Neutral red	5.64	2.45
Aniline blue (spirit soluble)	0.00	1.10

C. *Specific Gravity of Ethyl Alcohol-Water Mixtures.*—A check may be had on the alcohol content of alcohol-water mixtures by determining the specific gravity. This is a valuable and accurate method of determining whether the alcohol used in the final dehydration of sections contains small quantities of water. The following table gives the specific gravity of mixtures of ethyl alcohol and water at 15.56° C., referred to water at the same temperature (U. S. Department of Agriculture).

Specific Gravity	*% Alcohol by Volume*	*Specific Gravity*	*% Alcohol by Volume*
0.98660	10.0	0.8639	80.0
0.97608	20.0	0.8339	90.0
0.96541	30.0	0.8161	95.0
0.95185	40.0	0.8121	96.0
0.9344	50.0	0.8079	97.0
0.9136	60.0	0.8035	98.0
0.8900	70.0	0.7989	99.0
		0.7939	100.0

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

COLD VACCINES, Evaluation of, Diehl, H. S., Baker, A. B., and Cowan, D. W. J. A. M. A. **111**: 1168, 1938.

In a carefully controlled study of the value of three different vaccines which are recommended for the prevention of colds the subjects were cold-susceptible students at the University of Minnesota.

A "control group" was observed during each year of the study. Such groups were chosen at random from the students who applied for cold prevention treatment; the members were treated in exactly the same manner as those of the vaccinated group, and they believed throughout the period of the experiment that they were receiving vaccine. Sterile physiologic solution of sodium chloride was administered hypodermically as a control for the subcutaneously administered vaccine and lactose-filled capsules as a control for the vaccines administered orally.

One of the most significant aspects of this study is the great reduction in the number of colds which the members of the control groups reported during the experimental period as compared to the number that the same students reported for the previous year. In fact, these results were as good as many of those reported in uncontrolled studies which recommend the use of cold vaccines.

The group which received vaccine subcutaneously experienced an average of 25 per cent less colds per person than did the control group. This difference occurred during both years of the study and is statistically significant. Practically, however, it is of little or no importance, because a reduction of 25 per cent in the average number of colds in a group of individuals is not sufficiently great to justify the time and expense involved in carrying out the intensive vaccination procedure which was utilized.

The group which received the polyvalent vaccine administered orally experienced just as many colds as the control group during both years of the study.

The results reported by the students who took Rosenow's streptococcus vaccine parallel exactly those reported for the control group.

Although the data are not entirely conclusive, there is no evidence in this study either that vaccines reduce the complications of colds or that the condition of the nose and throat is related to the frequency of colds in a cold-susceptible group.

DIPHTHERIA TOXOID, Reactions Following Use of, Hayman, C. R. Am. J. Dis. Child. **56**: 723, 1938.

Reactions to the injection of undiluted plain toxoid are frequent and severe enough to warrant caution.

The age of the subject is a good guide in foretelling reaction.

The diluted toxoid test is of value in forecasting reaction in those younger than 15 years; the Schick control test is not. Both are of value in those older than 15 years.

Reactions are less influenced by change in dose of undiluted toxoid than by variation in age and cutaneous sensitivity.

Lack of reaction to an initial injection does not warrant giving a much stronger second injection.

The following scheme of immunization is suggested:

A. If the subject is 15 years of age or older:
   1. Perform preliminary cutaneous tests, using
      (a) Schick and Schick control test or
      (b) Schick and diluted toxoid (1:20) tests or
      (c) all three.

2. If the Schick test is positive, give the first injection as follows:

(a) If the Schick control test, the diluted toxoid test or both are negative, inject 0.5 c.c. of undiluted toxoid.

(b) If the highest reading is 1 plus, inject 0.25 c.c. of undiluted toxoid.

(c) If the highest reading is 2 plus, inject 0.1 c.c. of undiluted toxoid.

(d) If the highest reading is 3 plus, inject diluted toxoid or another material, such as toxoid-antitoxin floccules, or do not immunize.

3. For subsequent injection:

(a) If no reaction has occurred, give the same or, at most, a double dose.

(b) If a reaction has occurred, give the same or a decreased dose.

B. If the subject is under 15 years of age:

1. Do the preliminary cutaneous tests, using

(a) Schick test alone or

(b) Schick and diluted toxoid tests.

2. If the Schick test is positive, give the first injection as follows:

(a) If the diluted toxoid test was omitted, inject 0.1 c.c. of undiluted toxoid.

(b) If the diluted toxoid test reading is 1 plus, inject 1 c.c. of undiluted toxoid.

(c) If the diluted toxoid test reading is 2 plus, inject 0.5 c.c. of undiluted toxoid.

(d) If the diluted toxoid test reading is 3 plus, inject 0.25 c.c. of undiluted toxoid.

3. For subsequent injection, proceed as in A.

BLOOD GROUPING, Artificial Concentration of Test Serums in, Hoxworth, P., and Mahoney, E. J. A. M. A. **111**: 1554, 1938.

The lyophile method of drying serum as reported by Flosdorf and Mudd appeared to the authors as a possible method for accomplishing the same purpose with blood typing serum. They have been able to dry serum by this method and redissolve the residue in less than the original volume. The resulting product was found to have a hemagglutinin titer greater than the unprocessed serum and to be suitable for use as test serum.

TRICHINELLA SPIRALIS, Present Incidence of, as Determined by a Study of 1060 Unselected Autopsies in St. Louis Hospitals, Pote, T. B. Am. J. M. Sc. **197**: 47, 1939.

Muscle tissue from 1060 unselected autopsies were trichinous to the extent of 15.4 per cent. There had been no symptoms suggesting trichinella infestation.

Ninety-five per cent of the infestations were dead, calcified, and more or less disintegrated; 5 per cent were demonstrated to be alive by feeding the infested material to rats and recovering live trichinella larvae.

Very heavy infestations were to be observed where the individual gave a history of always having enjoyed good health. In one patient, dying at the age of 84, as the result of an accident, autopsy did not show that the infestation had contributed to the cause of death.

In 1500 hog carcasses examined for trichinella, 0.8 per cent were found to be infested, which gives rise to the question whether man is getting all of his trichinella infestation from pork. It may be concluded that man's infestation comes from pork derived from the small packing plants, which are not supervised by meat inspection services. It is to be noted that the infestations that sporadically occur are from pork from the establishments not under federal inspection.

It can be very definitely concluded that the *trichinella spiralis* infestation of man is not a serious lethal factor in Missouri, since in 163 instances of infestation it was not held to have been the cause of death or to have contributed to the cause of death.

ENDOCARDITIS, Bacterial, Mechanism of Localization of Vegetations of, Allen, A. C. Arch. Path. **27**: 399, 1939.

In from 50 to 75 per cent of cases bacterial endocarditis is superimposed on rheumatic endocarditis. Rheumatic endocarditis commonly produces a valvular fibroplastic deformity with stenosis. This lesion takes the form of a projecting shelf or barrier, usually at the line of closure, against which the blood stream strikes. By virtue of this obstruction to the systolic discharge (manifested by myocardial hypertrophy), the site of the deformity suffers a distinctly greater impact and contact than the normal valve leaflet, which "gives" or yields with the stream. This contact with the blood (and organisms in a bacteriemia) is further enhanced by the diastolic backflow due to insufficiency—the usual concomitant of stenosis. The role of these dynamics in the localization of vegetations is suggested.

This same mechanism applies to congenital lesions, including the "so-called congenitally bicuspid aortic valve."

Attention is called to the fact that the outflow surface of all valves, normal or deformed, comes in contact with a much greater area of blood (and toxic agents) than the opposite surface. The significance of this in the localization of vegetations is stressed.

An explanation based on the same principles is offered for the rarity with which auricular fibrillation is complicated by bacterial endocarditis. The possibility of the influence of other auxiliary factors is not precluded.

It is pointed out that there is an increased tendency for acute rather than subacute endocarditis to occur on (a) valves not previously deformed and (b) valves of the right side of the heart. This fact is correlated with the principles of impact and contact.

BLASTOMYCOSIS, Martin, D. S., and Smith, D. T. Am. Rev. Tuberc. **39**: 488, 1939.

From a study of the recorded cases of blastomycosis and their own series of 13 cases, the authors have drawn the following conclusions:

American blastomycosis (Gilchrist's disease) is a distinct clinical entity caused by a specific etiologic agent, *Blastomyces dermatitidis*.

Two clinical types of infection are recognized: (a) cutaneous blastomycosis, which proceeds as a chronic or subacute ulcerating process and which usually responds to treatment with iodides or radiation, and (b) systemic blastomycosis, a highly fatal disease, characterized by pulmonary infection and widespread distribution of lesions in the subcutaneous tissues, bones, joints, central nervous system, and internal organs.

The same fungus, *Blastomyces dermatitidis*, causes both types of infection.

The disease is far more common in males, and the natural habitat of the fungus is evidently in nature, although proof of its existence in the environment has not been found.

Antibodies can be found in the sera of patients who are heavily infected; they persist until death unless the infectious process is overcome or greatly reduced.

Some patients develop a condition of hypersensitiveness to the infecting fungus, and this allergic state diminishes in the terminal stages of the disease. The degree of hypersensitiveness can be estimated by skin tests and can be materially reduced by the repeated injections of minute doses of a heat-killed vaccine.

Potassium iodide is curative in some cases, but it is a dangerous drug to administer to patients who are allergic to the fungus.

In systemic blastomycosis iodide therapy should be started only after the state of hypersensitiveness has been excluded by skin test, or artificially reduced by therapeutic injections of vaccine.

TUBERCULOSIS, Culture Methods in the Diagnosis of, Whitehead, H. G. Am. Rev. Tuberc. **39**: 540, 1939.

A total of 215 specimens of various types were examined for tubercle bacilli by direct and concentrated smears, cultures on two tubes each of four types of media and animal inoculation. Concentration did not increase the number of positive specimens, but satisfactorily prepared the material for culture and injection into a guinea pig. Culture and animal inoculation increased the number found on smear.

Petragnani's medium showed more efficiency in promoting growth of tubercle bacilli and more resistance to contamination than did the media recommended by Loewenstein, Corper, or Petroff for isolation from contaminated materials.

A series of 190 specimens without animal controls showed a similar superiority of Petragnani's medium.

Greater efficiency of the culture method is obtained by using several different types of media in routine laboratory search for the tubercle bacillus. Available data do not warrant the substitution of the culture method for the animal method, but indicate that both should be used for the maximum reliability in laboratory diagnosis of tuberculosis.

ENDOMETRIAL BIOPSY, Clinical Interpretation of, Katz, J., and Parker, E. Endocrinology **24**: 447, 1939.

Three hundred endometrial biopsies from completely controlled patients in whom ovarian dysfunction was suspected have been studied and classified. Abnormalities which occurred in every stage of development have been described and interpreted in terms of disturbance of the functional level of the ovarian hormones.

Abnormalities of the endometrium reflect either failure of orderly elaboration or a disturbance in the normal balance of the two ovarian hormones at various times in the menstrual cycle. Pseudomenstruation, which is often considered to be synonymous with anovulatory menstruation, is not uncommon, occurring in 40 cases of this series.

Subnuclear vacuolization, which has been described as diagnostic of the occurrence of ovulation, is exceedingly variable in time of occurrence and may be superimposed upon an abnormal follicular type of endometrium. Secretory differentiation may be instituted in an endometrium showing evidence of either deficient or excessive follicular proliferation. The corpus luteum does not function in an "all-or-none" fashion, but it may elaborate an excess of one hormone (estrone) or a deficiency of one or both hormones.

There are no specific endometrial patterns for the gynecologic symptoms, with the possible exception of amenorrhea, in which a follicular endometrium is usually encountered. Pathologic bleeding may occur from any type of endometrium described. These same types occur in patients in whom there is no tendency to bleed. Menopause has no specific endometrial pattern. The endometrium merely reflects the degree of ovarian failure.

PNEUMONIA, Giant Cell of Infancy as a Manifestation of Vitamin A Deficiency, Chown, B. Am. J. Dis. Child. **57**: 489, 1939.

The hypothesis that the giant cells of giant-cell pneumonia represent metaplasia of alveolar epithelium due to vitamin A deficiency cannot be proved by argument. Proof or disproof must await experiment. So, too, only experiment can answer the questions of whether infection can cause either local or general depletion of vitamin A, and whether such depletion can be prevented by giving the vitamin during the course of infection. It is an interesting problem.

Five cases of giant-cell pneumonia are reported, and the possibility that the giant-cell formation is the result of vitamin A deficiency is discussed, though no conclusion is reached. The effect of infection on vitamin A depletion is also considered.

TULAREMIA, Diagnosis of, Friedenwald, W. F., and Hunt, G. A. Am. J. M. Sc. **197**: 493, 1939.

The results of diagnostic tests in 50 cases of tularemia are presented.

The blood agglutination is the most constant and reliable diagnostic method after the first week of the disease.

The antigen skin test is positive during the first week of the disease and appears to be highly specific and reliable.

The antiserum skin tests, both goat and horse, have been of value in only a small percentage of cases, because of nonspecific reactions of control sera. It is always necessary to use a control serum in this test.

The opsonocytophagic reaction tends to parallel the blood agglutination in time of appearance and is valuable in differentiating Brucellosis from tularemia, when cross agglutination occurs.

DERMATOSIS, Precancerous, and Epithelioma in Situ, Montgomery, H. Arch. Dermat. & Syph. **39**: 389, 1939.

In the group of precancerous dermatoses are included true Bowen's disease, senile keratoses, keratoses resulting from arsenic, tar, or radiation, and leucoplakia of the mucous membranes. In 20 per cent or more of the patients, epithelioma of varying degrees of malignancy eventually will develop, usually starting as squamous-cell epithelioma in situ. The conditions are all interrelated by their common histologic picture, and hence, a possible common carcinogenic factor. They are not to be confused with the relatively rare epithelioma in lesions of lupus vulgaris or other benign dermatoses, such as psoriasis and lichen planus. Precancerous dermatoses also must be distinguished from various conditions which result in the histologic picture of pseudoepitheliomatous hyperplasia. The incidence of epithelioma associated with lupus erythematosus or with scars from burns or other causes will vary up to 10 per cent in cases of such involvement, but the senile cutaneous changes, the age of the patient, the situation of the lesions, including the factors of repeated trauma and irritation, and the previous treatment employed must be considered. In many cases of precancerous dermatosis, histologic transitions between a benign and a malignant process may be seen in the same section.

The peculiar or distinctive and predominant histologic feature of epithelioma in situ with the phenomena of Bowen's disease, together with their relatively long duration as such, without clinical or histologic evidence of malignant invasion, justify the grouping of true Bowen's disease, senile keratosis, keratosis resulting from arsenic, tar, and radiation and in most cases leucoplakia of the mucous membranes under the title "precancerous dermatoses." If the term "precancerous dermatosis" is used in the limited sense defined, it has a definite meaning both clinically and pathologically and is of value from the standpoint of prognosis and treatment until more is known about the cause of cancer. The term "precancerous dermatosis," however, should not be used loosely when the pathologist is in doubt regarding the clinical or histologic picture, despite the fact that at present there is no general agreement regarding the distinction between cancer and precancerosis of the skin.

PAGET'S DISEASE, Extramammary and Intraepidermal Carcinoma, Pinkus, H., and Gould, S. E. Arch. Dermat. & Syph. **39**: 479, 1939.

A discussion is given of the nature and pathogenesis of the disease reported in the literature as extramammary Paget's disease.

Three cases are presented which offer, respectively, clinical, histologic, and clinical and histologic features of Paget's disease.

Criteria are discussed by which Paget's disease can be differentiated from similar diseases (Bowen's disease, erythroplasia, and superficial epidermoid carcinoma).

In accordance with the views of previous authors, Paget's disease of the nipple is defined as the expression of a more or less well-balanced symbiosis of the epidermis and an epidermotropic strain of cancer cells of mammary origin.

It is proposed to designate the interesting and unique biologic phenomenon of symbiosis of epidermis and "Paget cell" as the "Paget phenomenon."

On the basis of this biologic conception it appears justifiable to include as instances of Paget's dermatosis all those cases in which the Paget phenomenon is present, Paget's disease of the nipple being only its most frequent and best known example.

Extramammary Paget's dermatosis is due to the intraepidermal spread (a) of carcinoma of the apocrine glands (and perhaps of other cutaneous glands), (b) of carcinoma of mucous membranes bordering on the skin, and (c) possibly of melanoblastoma.

LAUGHLEN TEST, Inadequacy of the, and the Ide Test in the Diagnosis of Syphilis, Flood, J. M., and Mayer, V. Arch. Dermat. & Syph. **39:** 510, 1939.

A comparative study of the Laughlen and the Ide tests with the Kline, Kahn, and Kolmer tests is presented.

Tests were performed according to directions supplied by the distributors of the antigens.

The Laughlen and the Ide tests are less specific than the three standard tests used.

The Laughlen and the Ide tests are inadequate for the serodiagnosis of syphilis.

The Ide test is satisfactory for the examination of spinal fluid, but the Laughlen test grossly fails when the spinal fluid is not concentrated.

BLOOD, Preserved, Citrated in "Banks" in Relation to Transfusion in the Treatment of Disease, With Special Reference to the Immunologic Aspects, Kolmer, J. A. Am. J. Med. Sc. **197:** 443, 1939.

While sodium citrate is slightly anticomplementary, the complement of citrated (0.35 per cent) human blood, kept at 4° to 6° C., was well preserved for periods of from fourteen to twenty-one days. The same has been found true of undiluted citrated guinea pig complement, kept at 2° to 4° C.

The bacterial activity of normal citrated human blood, kept at 4° to 6° C. for *Staph. aureus*, beta hemolytic streptococcus, and *B. coli*, decreased after seven to twenty-one days' preservation.

The phagocytic activity of the neutrophiles of preserved citrated human blood, kept at 4° to 6° C. for *Staph. aureus*, beta hemolytic streptococcus, and *B. coli* was definitely reduced within seventy-two hours after collection of blood, becoming markedly so on or about the fifth day, followed by almost total absence of phagocytic activity on and after the seventh day of preservation. This may have been due to deterioration of normal opsonins but was mostly due to autolytic and degenerative changes in the leucocytes.

The erythrocytes of preserved citrated human blood, kept at 4° to 6° C., showed evidences of swelling and dehemoglobinization as early as forty-eight hours after collection, with progressive degenerative changes up to fourteen days, when at least 30 per cent were shadows, swollen and fragile.

The neutrophiles of preserved citrated human blood, kept at 4° to 6° C., showed evidences of disintegration with reduction in numbers as early as twenty-four hours after collection, with progressive disintegrative and autolytic changes up to fourteen days (longer intervals of preservation not being employed).

The platelets of preserved citrated human blood, kept at 4° to 6° C., showed distinct clumping immediately and twenty-four hours after collection, with evidences of deterioration in the latter. At the end of forty-eight hours they became scarce, and after five days only blue chromatin masses remained.

For reasons discussed it is believed that citrated human blood, preserved at 4° to 6° C., may be useful in the treatment of acute hemorrhage and shock for the purpose of restoration of volume, but is inadvisable for the treatment of the anemias, the blood dyscrasias or infections, and certainly should not be employed for these purposes after two to three days' preservation.

ANEMIA, Sickle Cell, The Erythrocyte in, Diggs, L. W., and Bibb, J. J. A. M. A. **112:** 695, 1939.

Little information is to be obtained from the literature concerning the sedimentation velocity of erythrocytes in sickle-cell anemia. The sedimentation rate was determined for 23 patients in our series. The total number of determinations was 49. Oxalated blood and 5 c.c. Cutler tubes were used; the readings were made at five-minute intervals for one

hour. In four instances the sedimentation rate was 5 mm. or less in one hour, and in 14 instances the rate was less than 10 mm. in one hour. Repeated examinations of the sedimentation rate for the same person at different times revealed variable rates, although the degree of anemia did not significantly change. Increased rates could usually be explained by some demonstrable abnormality, such as active tuberculosis, salpingitis, or badly infected ulcers of the legs, but in some cases there was no obvious cause for the increased rate. The fact that the sedimentation rate may be normal in spite of severe anemia indicates that the increased rate in these cases is due to some complication, whether it is clinically demonstrable or not. Since the sedimentation rate may be normal in the presence of marked anemia, it is obvious that correction factors based on the degree of anemia are not applicable in this disease.

TUBERCULOPROTEIN, Specific Effects of, Corper, H. J., and Cohn, M. L. J. A. M. A. **112**: 403, 1939.

Specific immunity against infections with virulent human tubercle bacilli could not be produced by Seitz filtrates from cultures of virulent human tubercle bacilli, by highly concentrated (ultradialysis) filtrates, or by the precipitated tuberculoprotein or alum-treated filtrates.

The highly concentrated filtrates, or precipitated tuberculoprotein, possess no appreciable primary toxicity for normal animals.

A primary intravenous injection of large amounts of normal Seitz filtrates, highly concentrated (by ultradialysis) filtrates or precipitated tuberculoprotein from these filtrates does not sensitize to a second intracutaneous injection with a fairly large test dose (0.1 mg.) of either filtrate or tuberculoprotein. A reaction to tuberculin can be obtained, however, in a tuberculous guinea pig with as little as 0.000005 mg. of the same material.

A primary intravenous injection of about 2 mg. of tuberculoprotein in any of the foregoing forms sensitizes guinea pigs to a lethal intravenous provocative dose (anaphylactically) of as little as about 1 mg. of the tuberculoprotein in these forms. This confirms the studies of Zinsser, who worked only with large amounts of bacillary extracts and showed a striking difference between cutaneous and anaphylactic reactions.

To produce cutaneous hypersensitiveness to tuberculoprotein requires a small amount of avirulent human tubercle bacilli (a tubercle-forming dose), a large amount of heat-killed tubercle bacilli (around 100 mg.), and very small amounts of virulent tubercle bacilli. With the latter, the factors of multiplication and tuberculous involvement complicate the picture.

There is a striking quantitative difference between the specific immune and the concomitant allergic or anaphylactic features of tuberculosis which will eventually have to be given independent consideration in the complete evaluation of tuberculosis in man, since one is protective and the other shows a peculiar type of still unsolved intoxication.

BONE MARROW: Sternal Marrow of Children in Normal and Pathologic States, Vogel, P., and Bassen, F. A. Am. J. Dis. Child. **57**: 245, 1939.

The following method was used for the study of sternal marrow:

The skin over the upper portion of the sternum was prepared with iodine and alcohol. The site usually selected for puncture was at the level of the upper border of the third rib in the midline of the body of the sternum. In infants and in young children, however, the manubrium was found to be preferable for aspiration, the reason being that this portion of the sternum is thicker and, therefore, safer for penetration, at the same time tending to fix the needle more firmly. No anesthesia was necessary. The sternal plate in children is so thin that pain is only momentary. A ½ inch (1.27 cm.), 18 gauge lumbar puncture needle was inserted into the marrow cavity at an angle of about 45 degrees, with the point directed cephalically. For children under 1 year of age a 20 gauge needle of ¼ inch (0.64 cm.) length is preferable.

EFFUSIONS, Malignant Cells in Serous, McDonald, J. R., and Broders, A. C. Arch. Path. 27: 53, 1939.

Examination of the sediments obtained from effusions is of definite value in patients in whom malignant growths involving serous cavities are suspected.

Smears made from the sediments of fluids are as efficacious in studying cellular detail as paraffin sections. Cells with eccentric nuclei and clumped cells are of little value when one is determining the nature of serous effusions.

When malignant cells can be identified definitely in serous effusions, the diagnosis of malignant growth carries a high degree of accuracy.

The technique used in examination of these specimens was simple. From 20 to 40 c.c. of fluid was centrifuged for fifteen minutes at approximately 1,800 r.p.m. When possible, the last portion of fluid withdrawn from the cavity was chosen, although frequently a choice could not be made. The fluid was centrifuged immediately after removal, because the clot which forms after standing interferes with this method of examination. The centrifugate was smeared rather quickly on clean glass slides and was allowed to dry in air. The slides were immersed in solution of formaldehyde U.S.P. (diluted 1:10) for twelve or more hours. The smears then were stained with hematoxylin and eosin, carried through the alcohols, carbolxylene and xylene, and mounted in Canada balsam.

Entrance into the marrow cavity of an older child was recognized when there was a sudden give of the needle, which indicated the penetration of the outer table of the sternum. A tight-fitting, dry, 5 to 20 c.c. syringe was attached to the needle after the stilet was removed, and about 0.1 or 0.2 c.c. of the marrow fluid was withdrawn; counts of the nucleated cells and one of the megakaryocytes were then made from this drop, using a 2 per cent solution of acetic acid. This technique minimized the admixture of blood and trauma of the marrow cells. The characteristic suction pain was almost negligible. The smears were stained with a combination of the Jenner and the Giemsa methods, and a differential count of at least 500 nucleated cells was made. Whenever possible small fragments of marrow were teased out with a Hagedorn needle and gently spread across the slide. Study of these thick smears furnished a better idea of the distribution and cellularity of the material aspirated. In addition, a small quantity was placed in a small test tube and allowed to clot. When the clot was firm, Zenker's solution and 2 drops of glacial acetic acid were added. After two hours of fixation, the clot was washed with tap water and placed in 70 per cent alcohol. Sections were made in the usual manner. When chips of bone were present, the stained sections revealed tiny islands of marrow enmeshed in the clot. Sections made in this fashion are not as representative as those obtained by a regular surgical procedure, but they generally serve the purpose.

Observations on the marrow of 41 normal subjects and of 72 subjects with various types of blood dyscrasia and with other diseases are presented.

The sternal marrow is of diagnostic and confirmatory value in certain blood dyscrasias; in other instances it may be helpful in ruling out the presence of dyscrasia.

ITEMS

Institute for the Consideration of Blood and Blood-Forming Organs

The University of Wisconsin Medical School will conduct an Institute for the Consideration of the Blood and Blood-Forming Organs, September 4-6, 1939. The program will include papers and round table discussions by European and American workers in the field of hematology. In addition to the discussions, the following formal papers will be presented:

Dr. L. J. Witts, Oxford, England, Anemias Due to Iron Deficiency.
Dr. Cecil J. Watson, Minneapolis, The Porphyrins and Diseases of the Blood.
Dr. Cornelius P. Rhoads, New York, Aplastic Anemia.
Dr. E. Meulengracht, Copenhagen, Denmark, Some Etiological Factors in Pernicious Anemia and Related Macrocytic Anemias.
Dr. Harry Eagle, Baltimore, The Coagulation of Blood.
Dr. George R. Minot, Boston, Anemias of Nutritional Deficiency.
Dr. Russell L. Haden, Cleveland, The Nature of the Hemolytic Anemias.
Dr. Jacob Furth, New York, Experimental Leucemia.
Dr. Claude E. Forkner, New York, Monocytic Leucemia and Aleucocythemic Leucemia.
Dr. Edward B. Krumbhaar, Philadelphia, Hodgkin's Disease.
Dr. Louis K. Diamond, Boston, The Erythroblastic Anemias.
Dr. Edwin E. Osgood, Portland, Marrow Cultures.
Dr. Charles A. Doan, Columbus, The Reticulo-Endothelial System.
Prof. Hal Downey, Minneapolis, Infectious Mononucleosis.
Dr. Paul Reznikoff, New York, Polycythemia.

Physicians and others who are interested are cordially invited. A detailed program may be obtained by addressing Dr. Ovid O. Meyer, Chairman of Program Committee, University of Wisconsin Medical School, Madison, Wis.

Biological Photographic Association Convention

The Ninth Annual Convention of the Biological Photographic Association will be held September 14 to 16, at the Mellon Institute for Industrial Research, Pittsburgh, Pa. The program will be of interest to scientific photographers, scientists who use photography as an aid in their work, teachers in the biological fields, technical experts, and serious amateurs. It will include discussions of motion picture and still photography, photomicrography, color and monochrome films, processing, etc., all in the field of scientific illustrating. Modern equipment will be shown in the technical exhibit; and the Print Salon will display the work of many of the leading biological photographers here and abroad.

The Biological Photographic Association was founded nine years ago because of the growing need for expert illustrative material for scientific research and teaching. Many workers were solving their problems in their own way. But obviously they were wasting their time and effort in individually repeating experiments that had been worked out elsewhere. The B.P.A. was formed to act as a clearinghouse for new ideas, to pool experiences, to record standard procedures, and to disseminate information. Its aims were scientific, and all services have been volunteered by officers and members on a nonprofit basis.

The B.P.A. Journal is published quarterly, constituting a volume of about 250 pages, and is furnished free to members. Membership privileges include an authoritative question and answer service; also the right to borrow loan albums and exhibits of scientific prints for study and display.

Further information about the Association and the Convention may be obtained by writing the Secretary of the Biological Photographic Association, University Office, Magee Hospital, Pittsburgh, Pa.

CORRESPONDENCE

Boise, Idaho,
May 20, 1939.

The Editor,
JOURNAL OF LABORATORY AND CLINICAL MEDICINE.

Dear Sir:

Page 891 of your May, 1939, publication contains an abstract of an article by A. E. Platt entitled "Salt Optima in Agglutination," with special reference to *Brucella abortus*, in which the author states that the antigen-antibody optimal ratios remained constant for all salt concentrations between 2M/7 and M/56. In this connection, it may be of interest to you to know that in Bang's disease agglutination tests performed in this laboratory a 50M/58.5 concentration of sodium chloride was found to give the most complete agglutination, that is to say, no hazy reactions. When this concentration of salt is combined with 0.02 c.c. of a 1 per cent solution of brilliant green and 0.02 c.c. of a 1 per cent solution of gentian violet per 100 c.c. of the salt solution, a very satisfactory antigen for Bang's disease is obtained.

HARRY H. UTES
State Department of Agriculture